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ABSTRACTS
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Potato for a Changing World

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Dear participants to the 17th Triennial Conference of the EAPR,

Dear guests, dear friends,

I am very pleased to extend to you all a cordial welcome to the Conference, welcome to Romania and to the city of Brasov.

On behalf of the Council of the European Association for Potato Research and the Organizing Committee, I would like to thank you for joining this Conference which is held in the UN International Year of the Potato and one year after the 50th EAPR anniversary.

In a world confronted with climate shock, energy and food crisis, our potato is still the most important tuber crop and it is expected from it to provide food security and to support the achievements of Millennium Development Goals.

The triennial conferences organized by the EAPR are interactive forums where scientists and potato experts meet and share their thoughts, identify solutions for a "potato in a changing world" and built new bridges between the various links in the chain.

We will have some busy days here and authors will give fascinating presentation in their own activities.

There are 345 participants from 42 different countries, 15 plenary lectures, 72 oral and 145 poster presentations. Four workshops, three scientific excursions and some business meetings have been arranged.

In the last three years since Conference in Bilbao in 2005, many aspects of potato research have been covered and now it is time to resume the progress done and to look ahead into the future.

Please allow me to mention just a few: -genetic modification is going to be a great practical success - the entire genetic chart of the potato will have been mapped out in two years; - marker-driven breeding and in-vitro techniques.

At the same time organic market is getting very important and the consumer of tomorrow demands us to be more focused on health, environment and sustainability.

We have the opportunity to meet many colleagues from Western and Eastern Europe and from non-European regions and we will learn about their specific approaches and problems of potato chain.

Let hope that the Conference will convince potato community about the challenge and the fantastic job we have be able to do for potato in a changing climate.

During these days, the combination between scientific, technological and cultural events allows you to see more of the Romanian lifestyle.

Thanks to the colleagues and to all who have participated in the preparation of this conference.

I am sure that you will have a very fruitful and successful time in Brasov Conference and in Romania.

Sorin Chiru
President of the EAPR

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Key notes

HISTORY, STATISTICS AND TRENDS IN ROMANIAN POTATO INDUSTRY

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Historical references

Potato has a relatively long tradition in Romanian agriculture, first references being made in Transilvania in the XVIII Century, when in 1760 The "Practical Knowledge for Potato Crop" was published (Morar. 1999)..

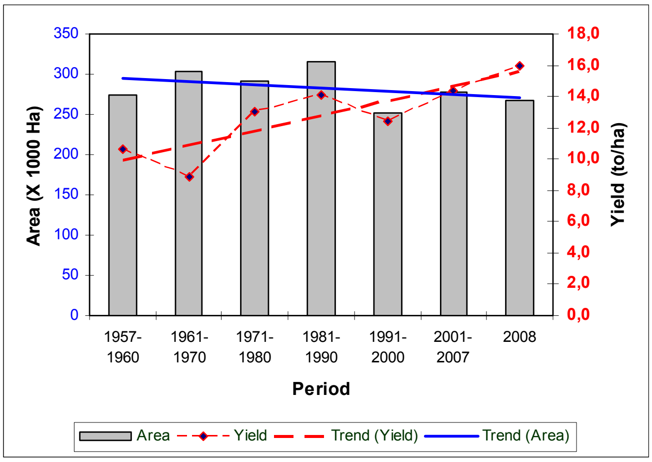
Like in other European countries the big famine of 1800 contributed to the spread of potato in all three Romanian provinces (Moldavia, Transilvania and Walachia). The different names kept for potato in Romanian language, certify the origin zones, the majority being in Germany and Austria.

With the passing of time the crop importance increased and potato is now considered the "second bread" of Romania.

Statistics and trends

Except for the years 1970-1990 when the surfaces were concentrated on an industrial system of cultivation, in both earlier and present periods in potato crops the small plots dimensions prevailed.

During the last 50 years, the average surface was 250000-316000 ha (placing Romania on 2nd-3rd spot with Germany and after Poland) with an average yield of 14.5 t/ha and a total yield of 2.6-4.4 million tones (Figure 1.)



Source: FAO Database, 2006 and MARD, 2008
Figure 1. Evolution of potato area and average yield in Romania in the last 50 years (1957-2007) and 2008

Compared with an average yield of 30-40 t/ha in Western countries in the respective period, the yield in our country was 2.5-3 times smaller, determined by some restrictive factors (Chiru et al., 2006):

- reduced size of agricultural exploitation (more than 2 million landowners have less than 0.3 ha (Table 1.);
- phyto-sanitary qualities of planting material;
- lack of financial resources for potato growers;
- low professional level of potato growers;
- climatic conditions of last years (Olteanu et al., 2008).

Agricultural exploitation type	Number of landowners	Potato surface average (ha)
Individual growers	2261000	0.3
Family associations	1197	13.7
Companies	498	25.6
Research-development units	5	86.0

Source: MARD, 2008
Table 1. The reduced sizes of land areas (2007

An analysis concerning Romanian positioning against some Central and East-European countries, in 1996-2006, reveals that, after Poland our country occupies second place like surface and total yield. A general characteristic is the fact that the surfaces remained on crop are in 2006 about 49-68%, except Romania with 95% surfaces with crops. The same reduction tendency was on total potato yield (Table 2).

Country	Area (ha x 1000)				Total Yield (t x 1000)			
	Average 1996-06	2006	Difference	%	Average 1996-06	2006	Difference	%
Poland	1112.39	597.23	515.16	54	20008.46	8981.98	11026.48	45
Romania	**299.38**	**283.09**	**16.29**	**95**	**4155.01**	**4015.90**	**139.11**	**97**
Czech R.	61.17	30.03	31.14	49	1309.05	692.17	779.02	53
Hungary	46.16	22.58	23.58	49	991.58	574.44	417.14	58
Bulgaria	44.94	24.47	20.47	54	523.74	386.05	137.69	74
Slovakia	29.56	18.38	11.18	62	464.21	263.08	201.13	57
Slovenia	8.73	5.92	2.81	68	179.02	106.97	72.05	60

Source: FAO Databases, 2006 and MARD, 2008
Table 2. Evolution of potato area and total yield in Romania in seven Central and Eastern countries (1996-2006) and 2006

Results of potato research.

In the Brasov area, potato research has a long tradition justifed by favourable ecological conditions, due to the importance of this crop for food, industry and animal fodder.

Potato research started in Romania after the foundation in 1927 of the Agricultural Research Institute ICAR. In 1967 The Research Institute for Potato and Sugar Beet Brasov (ICCS) was set up, an event which marked the beginning of modern research for potato. 2007 was the year in which 40 years of uninterrupted activity of the Institute were celebrated.

Between 1920 and1950, the importance of potato breeding started the research for the identification of best varieties from world range (Chiru et al., 1992, 2007). First varieties were created at Bod by Stephani, starting with 1923 (Maikoning, Edelrosen). Velican created the Ardeal, Someşan and Napoca varieties. between 1930 and 1945.

An important qualitative leap in potato breeding activity was between 1951-1966, after improving selection methodology and application of breeding restrictive criterions conc erning the resistance to late blight, wart disease, virus diseases (Constantinescu et al., 1969).

Thus, between 1950 and 1966, there were created the following potato varieties: Bucur, Colina, Poiana, Padina, Ghimbăşan, Măgura, Carpatin and Braşovean During the period 1967-2000, another 30 potato varieties were obtained, the most representative being: Semenic, Super, Mureşan, Rustic, Roclas, Nana, Christian, Nicoleta, Redsec, Rozana, Robusta and Sucevița. These varieties, characterized by superior performance were the result of intensification in breeding activity, both at the Institute and Miercurea Ciuc, Târgu Secuiesc, Suceava research stations (Bozeşan, 2002). During 2000-2007 there were created 25 new potato varieties, with proper qualities for different tasks. A special attention was given to precocity, dynamics of tuberization and ecological plasticity. Thus, Dumbrava, Transilvania, Magic, Astral, Claudiu, Luiza, Milenium, Rozal and Amicii varieties were created.

Settling potato degeneration zones and production of varieties for different purposes was an important research aim. Based on these results closed zones for potato production according with climatic conditions and production purposes (early potato, summer potato, autumn-winter consumption, seed potato and processing) were created. Clearing up the potato degenerative phenomena allows us to establish theoretical and practical bases to create the national system of seed potato production. This system ensured the necessary planting material from internal production till 1990 (Draica et al., 1992).

To increase the safety of potato viruses diagnosis (especially with PLRV, PVY and PVA infection), ELISA technique was introduced and extended, starting with 1998.

Taking into consideration that potato pests and diseases resistance is an important element to decrease yield losses, an important activity was done to elaborate new testing methods for late blight resistance, on the field, greenhouse and laboratory. Potato varieties bred in our country had field resistance comparable with those of foreign varieties. Due to new qualitative standards, other pests were studied (wireworms, potato rot nematodes, potato cyst nematodes) which affects potato yield quality (Plămădeală, 1989). The results from 1967-2007 were materialized in 380 breeding lines, over 40 new potato varieties, 130 technologies and modern methodologies, through 2000-2400 t/year of biological material from superior categories.

Present and future objectives

To solve the problems of Romanian potato crop in the present economic and climatic conditions, the scientific research has the following objectives:

1) Development of potato breeding and genetic researches through new genomic and technological approaches, to obtain genotypes, which correspond to present and future requirements regarding the production of healthier foods, with high qualities, suitable for ecological agriculture and which permit utilization of new protective technological methods more friendly for environment, with conservation potential to regenerate natural resources.

The ways to solve this problem are the following:

- improving yield qualities, increasing diversity and breeding realizing new genotypes with special characteristics (antioxidants, vitamins, essentials);
- obtaining stress resisting genotypes to reduce the effects of adverse conditions (climatic changes, CO2 concentration, pollution);
- breeding the genotypes suitable for processing;
- breeding the genotypes suitable for organic crop.

2) Development of clean technologies types "from farm to fork" in accordance with sustainable agricultural principles, increasing food and safety security, respecting general and specific requirements of the market.

Directions to solve these problems are:

- perfecting the technologies and drawing up the Guide for Good Agricultural Practices (GAP).
- elaboration of support systems for decisions (DSS) for all involved processes (production, pest and diseases management, farm management).
- promoting the principles of Precision Farming through organizing an Excellency Centre (based on the newest scientific realizations:,intelligent and cognitive systems for monitoring, modelling and management of the processes from potato agro-ecosystems).

3) Promoting alternative technologies for potato crop to obtain ecological products.

Elaboration and promoting specific technologies for ecological crops with high environmental protection.

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THE INFLUENCE OF CLIMATE CHANGE ON POTATO DEVELOPMENT, GROWTH AND BIOTIC AND ABIOTIC FACTORS

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Summary

Since the onset of the industrial revolution the amount of carbon dioxide rose from 290 to 380 parts per million. Especially the last decennia, the warming effect of more greenhouse gases is being felt. The last fourteen years worldwide contained the warmest thirteen years since weather recording started. For Europe the major effects are the following. The dry and warmer southern Mediterranean parts will be come hotter and dryer still. The temperatures will continue to rise with a decreasing number of days with frost in northern Europe. The mean daily minimum temperature in Europe moves up one degree. Rainfall in southern Europe decreases and in the North-West it increase in winter. In summer there will be more dry spells but also more periods with extreme rainfall. The main effects are shown in Box 1.

Box 1. Main effects of climate change expected for Europe (IPCC, 2008)

Winters and summers get warmer
Longer growing season
Winters get wetter
Rain falls less and shorter but more intense
Rivers flood (Poland, Netherlands, UK)
More rainy days in winter, less in summer
Increased precipitation deficit in summer, more drought
More extreme weather patterns
More sunshine
More CO_2 in the air
Mediterranean area: very hot and dry

The effects for potato are becoming clear. In the southern Europe potato production becomes problematic due to water shortage and too high temperatures leading to low yields and tuber dry matter concentration. In northern Europe yields will go up because of the longer growing season which allows earlier planting and a more rapid initial foliar development but also because higher concentrations of carbon dioxide in the air that speed up growth and thereby improve the efficient use

Box 2. Effects of climate change on potato yields

In Northern parts of Europe:
- Warmer springs, earlier snowings, longer season: higher yields
- Increased canopy development: more water and nitrogen: higher yields
- More carbon dioxide in the air: higher yields
- Stomata close a bit: less transpiration, improved water use
- On clay soils: soil structure worsens: lowers water availability

In Mediterranean areas
- Spring season shortens: lower yields
- More winter crops: lower yields

Box 3. Effects of climate change on potato quality

- Increased tuber size
- Increased Vitamine C, lower concentration of glycoalcoloids
- Increased dry matter concentration
- More hollow hearts
- More secondary growth and sugar ends
- Winter temperatures up: reduced storability
- More rotten tubers due to water logging

of water. Box 2 shows the repercussion of climate change on expected crop yields, The quality (Box 3) of the produce is going to be effected as well as a rainy period after a heat wave leads to secondary growth, knobbly tubers, glassiness and sugar ends in processing. Too high temperatures in winter make cooling with ambient air less successful and lead to more losses from respiration, sprouting and rot.

Box 4. The effects of climate change on potato pests and diseases

- More groundkeepers reduce rotation effect
- Earlier appearance of (late) blight
- Blight control: risk of run off, less field accessibility
- More aphids (negative for seed) and wireworm
- Increased pressure of Q-diseases: brown rot, ringrot
- *Erwinia* spp: *Pectobacerium* and *Dickeya* increase
- Colorado beetle moves North
- More cycles of nematodes

Higher temperatures – however - have unfavorable effects, especially on pests and diseases (Box 4). The Colorado beetle rapidly moves North and other insects become more harmful too such as virus transmitting aphids. This makes seed production more costly as it leads to a reduction of the number of generations in the field. Bacteria of which the development is also correlated with higher temperatures give rise to a build-up the pressure of quarantine diseases such as ring rot and brown rot and an increased incidence of Erwinia. The effect on late blight cannot be predicted with certainty: dry hot spells will decrease epidemics but intermittent very wet periods reduce fungicide efficacy, diminish field accessibility for spraying machines and washing of spores in the soil lead to more tuber blight. Blight epidemics start earlier and where mild winters increase the number of ground keepers the pressure from this source of inoculum augments.

To adapt to the new situation the potato industry will have to invest in the development of new potato production areas (further North), in new varieties adapted to extremes in weather (heat, drought), in irrigation equipment, in equipment better adapted to wet soil conditions to assure accessibility and improved stores with more stores equipped with refrigeration as higher winter temperatures more frequently will make it impossible to keep ware potatoes cool with ambient air.

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THE POTATO – HEALTHY OR NOT?

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We should reflect our daily diet, because nutritionists describe a general trend toward overweight and obesity in many countries. The result is, that several food categories have come under discussion – and potatoes belong to them. This contribution will balance the different aspects, but it is to accept as a basic principle that food itself cannot be healthy or unhealthy. Our behaviour is rather decisive and often we do not follow the official guidelines.

Potatoes are available all around the world, however the specific acceptance is different between countries and population subgroups (e.g. social classes and age groups). Next to freshly boiled or steamed potatoes, the consumers request more and more for potato products.

Updated evaluations of the different food categories by nutritionists have resulted in a downgrading of potatoes and potato products. Nevertheless potatoes have a nutritionally favourable composition with respect to several nutrients. Recently, the obtained knowledge of food borne toxicants and food contaminants has extended the discussion about healthy nutrition. Last but not least, rational aspects are always escorted by social and mental aspects, which make it more complicated in the right choice of a so called healthy diet. The consumers will even enjoy their food.

A view upon the composition of different potato foods clearly points out, that boiled potatoes are a good source of protein and carbohydrates, but with regard to the high water content, the nutrient density is low. Minerals and vitamins are valuable constituents. For instance a typical meal with 200 g of boiled potatoes (at least 3 medium sized tubers) will contribute to our daily diet

6 % of energy (Guideline daily amount GDA: 2300 Kcal),
0.3 % of fat (GDA: 70 g),
11 % of carbohydrates (GDA: 270 g),
9 % of protein (GDA: 50 g),
11 % of dietary fibres (GDA: 25 g),
47 % of vitamin C (Recommended dietary allowance RDA: 60 mg),
8 % of vitamin B2 (Riboflavin) (RDA: 1.2 mg).

Furthermore several minerals, e.g. potassium, and secondary constituents are relevant.

Processed potato foods like French fries are well accepted worldwide. Especially potato crisps have started a triumphal procession around the world. Today, there are only very few countries without crisps consumption. This snack food offers a sense of being, especially by its special taste. Of course it is not a basic food.

Consuming potatoes or potato food we should bear in mind several aspects reflecting healthy or even unhealthy tools.

Carbohydrates

Available carbohydrates are present both as starch or free sugars, whereas non-available carbohydrates are counted among dietary fibres. Concentration of starch varies widely with respect to genotype and environment. Typical ranges are between 10 and 20% starch in fresh weight. Raw potato starch has a high amount of non-digestible units, on the other hand gelatinized starch is easily available and the content of resistant starch in potato food is prevailing low. Sugars may be involved in heat induced reactions (e.g. Maillard reaction) resulting in adverse effects.

Downgrading of potatoes was especially performed with view to the glycaemic behaviour, because all the available carbohydrates can be absorbed as monosaccharides and metabolized by the body. As a consequence a postprandial blood glucose response is elicited. Potato carbohydrates are relatively rapidly available. Therefore, the chance in blood glucose concentration is fast and the glycaemic response is high. The resulting glycaemic index (GI) describes the relationship between food borne blood sugar and reference increase (glucose or white bread). Typical GI-values of boiled potatoes are between 20 and 100, to rank potatoes as a food with medium to high GI. The overall insulin mediated glucose uptake is based upon the glucose level in consequence of the carbohydrate concentration in a food. The relevant concentration in potatoes is almost low. Therefore, the term of

glycaemic load (GL) is to be followed combining GI and concentration of carbohydrates. GL of potatoes has a low or middle profile. Several nutrition guidelines were developed reflecting the GI or GL of single food (e.g. low glycaemic index pyramid, LOGI; healthy eating pyramid). Potatoes at the top of those pyramids guide to a sparely consumption, but the reason for that grading is based upon U.S. preferences of convenience food consumption (esp. French fries, baked potatoes, potato mash). The availability of GI-data does not reflect the high variance of carbohydrates in potatoes.
Next to *in vivo* measurements of the GI also *in vitro* techniques were developed to measure the rapidly and slowly available glucose. GI is not a stable feature of a food, but is considerably modified by its processing, composition, preparation, the composition of a mixed food and many other factors which renders it difficult to precisely predict the actual blood glucose increase in an individual situation. GI of potatoes may be changed dramatically by parallel consumption of proteins and fats. A high intraindividual variability exists in response to a carbohydrate load.
Furthermore, the debate on low carb food has enclosed potatoes. The absolute concentration of carbohydrates is low again. If consumers want to select low carb food, they may choice hard cooking potatoes.

Resistant starch (RS) is not available for human digestion, but fermentation towards short chain fatty acids (SCFA) takes place. RS level in prepared potatoes is low. Some increases arise in a longer stay after preparation, e.g. par fried French fries and potato salad. Raw potato starch has a very high level of RS – but only few people are going to eat raw potatoes.

Vitamins

Vitamins of the potato are well known to contribute substantial amounts of the recommended daily intake. Especially vitamin C, which is present as L-ascorbic acid (L-AA) and as mono-dehydro ascorbic acid (MDHA), has been studied intensively.
The biological function of L-AA is focused on the antioxidant properties. In humans, both forms, L-AA and MDHA, are active. MDHA can be reduced to L-AA. The plasma level of L-AA in large sections of the population is sub-optimal. Therefore, the L-AA level of plant food should be improved or losses may be minimized.

Following the post-harvest period, the sum of L-AA and MDHA declines, because some MDHA molecules convert to dehydro ascorbic acid (DHA), which undergoes decomposition toward 2,3-diketo gulonic acid, which is no longer active. The preparation itself indicates high losses by warming potatoes over. Several constituents are heat sensible. Leaching is also a reason for a reduced concentration.

Antioxidant capacity

Several secondary constituents of the potato have an antioxidant activity, which contributes to the physiological defence against oxidative and free-radical-mediated reactions. Next to vitamin C also other substances, e.g. flavonoids, carotinoids, and several phenolic compounds, are involved, but our knowledge about potato antioxidants is still low. Anyway, a diet with antioxidant rich food results in a high antioxidant level in the blood, which will reduce the incidence of degenerative diseases like cancer and arteriosclerosis. Data are often available from raw potatoes. Different levels of antioxidants are reported depending on flesh colour (e.g. dark yellow or blue). Generally, the preparation of potatoes will lower the antioxidants, long heating (e.g. boiling) reduces more than short preparations (e.g. microwave heating, frying). An average remaining capacity of 75% is described. The storage has also an influence (lit 1611). An increase during long term storage especially in case of a lost dormancy was noticed. Experiments with bread crust have shown a *de novo* synthesis within the Maillard reaction. Therefore, also fried potato products may have enhanced values in relation to boiled tubers. In comparison with legumes and fruits, potatoes offer a low but substantial contribution to the daily requirement. Methodical studies have shown, that potato antioxidative capacity is often undervalued with respect to the slow release of phenolic substances.

Heat induced toxicants

In the last few years, acrylamide has been intensively investigated with regard to findings in several food categories, fried and baked potato products included. It is mainly formed in food by the reaction of asparagine with reducing sugars as part of the Maillard reaction. Acrylamide is neurotoxic.

Furthermore, experimental studies with animals have shown reproductive, genotoxic, and carcinogenic properties, but a final evaluation of acrylamide occurrence in food is still missing. Therefore, appropriate efforts to reduce acrylamide concentrations in food should continue. Since 2002, most of the European potato food processors have reduced levels substantially. At the moment, private cooking techniques are the most critical point (e.g. finishing of French fries). Reduction measures for potato food are available.
Latest data have shown, that bound 3-monochloropropane-1,2-diol (3-MCPD esters) is present in some fried potato products. The potato itself is not the origin rather the refined frying oil. At the moment the amount of release during human digestion is still unclear.

Others

Potato tubers have the highest satiety index of all plant food. Furthermore, serious anti-nutritional reactions may occur, however, if raw or inadequately heated potatoes are consumed.

Potato tubers contain several protease inhibitors that inhibit the activity of trypsin, chymotrypsin, and other proteases, thus decreasing the digestibility and the biological value of the ingested protein. The concentration of trypsin inhibitors (TI) can be as high as 174 mg g^{-1} protein. Assuming a protein content of 2% FW in potato tubers, this may result in a TI content of up to 3.5 g/kg potato tubers. Also some potato lectins are present, but both anti-nutrients are largely inactivated by boiling and other thermal processes.

Conclusion

Potatoes offer several nutritional benefits and only few adverse ones. Unfortunately, most people have a limited knowledge about all these facts. Education campaigns could be helpful to promote the positive preparation types, and to increase the daily potato uptake.

Further readings

Carbohydrates

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POTATO SCIENCE FOR THE POOR

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Potato production and consumption in developing countries now exceeds that of industrial countries. During the last decade, developing country production has grown while that of industrial countries has declined, continuing a trend over the last two decades. Growth of production in China and India explain a large percentage of total production growth while the countries of Sub-Saharan Africa (SSA) doubled production during the last decade. Production growth is explained principally by producer response to market demand growth from urban consumers. These trends are expected to continue within the context of the food price crisis. Continued development of the potato market in developing countries represents overlooked opportunities for food security and poverty alleviation.

IMPORTANT THREATS IN POTATO PRODUCTION AND INTEGRATED PATHOGEN/PEST MANAGEMENT

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Among all agrophages, plant pathogens and pests affect significantly the size of yields and play an essential role in agricultural production. Each arable crop has its own particular "enemies", which occurs more frequently than others. Only its efficient control can provide the best conditions for high and healthy yield. Plant protection aiming at prevention of pest occurrence and suppression of disease development faces various problems for instance, pathogen variability often is affected by biological and environmental factors.

Since 1970's climate in Europe warmed rapidly. Over the next years climate is expected become gradually milder and more humid in N. Europe and hotter and more dry in the South. In Central Europe is becoming warmer with more extreme weather events (Kaukoranta, 2007). These changes are forecasted to be responsible for major changes in the distribution of plant and animals species, pathogen/pest prevalence and their biodiversity. On the other hand, some changes in pathogen' variability and epidemiology is affected by biological factors. Very good example of influence both of them is *P. infestans,* causing late blight (LB), which is still the greatest disease threat to potato crops.

An increase in infection potential of *P. infestans* caused by genetic changes in pathogen population and higher infection pressure were observed all over the world over last years (Spielman *et al.* 1991, Fry *et al.* 1993, Sujkowski *et al.* 1996, Hermansen *et al.* 2000). These changes result in earlier outbreak of LB and higher disease rate despite conditions not always favoring infection occurrence. LB spread is favoured by periods of warm (12-18^{0}C), wet weather and high humidity. Association with humid factors is especially seen if the first infections start on stems, as stem blight. The authors (Rowe, 1996; Bain *et al*., 1996) working mostly on stem blight point out the importance of stem lesions as a source of inoculum for the infection of progeny tubers. If weather conditions turn dry and hot, leaf lesions expansion stops, and the fungus does not develop. Lesions on potato stems stay active during the growing season and once favorable conditions appear the disease again develops.

Early blight (EB) caused by two species of genus *Alternaria: A. solani* and *A. alternate,* occurs commonly worldwide on potato crops, particularly in regions with alternating wet/dry periods, which favour spore formation and disease development provided by dew and/or irrigation and irrigated potato soils, light-textured, sandy, low in organic matter (Gudmestad & Pasche 2007). Both *Alternaria* species differ also in some morphological features such as mycelium color and mycelium growth rate on the media, spore structures and temperature requirements. In the course of disease development, the morphological symptoms are difficult to distinguish therefore they are evaluated jointly as the EB. The casual agents of EB are an example of typical necrotrophic organism i.e. a pathogen infecting weaker and older plants (Rotem 1966), infected with some viruses (Hooker 1990).

Similar environmental factors favour the incidence of *Colletotrichum coccodes*, causing black dot on potato plants The pathogen often invades plants that are weakened by other diseases, and it may speed up earlier death of haulm infected with *Verticillium, Pectobacterium* and even *Ph. infestans.* Black dot occurs most frequently on plants grown in coarse-textured soils under conditions of low or excessively high nitrogen, high temperature, or poor soil drainage.

Appearance of pathogens, such as *Pectobacterium (Erwinia) chrysanthemi,* known earlier only from warm region of N.Africa or Mediterranean zone is observed. *Ech* is responsible for increasing incidence of black leg in Central and northern Europe. In 2005, when temperature was high during the growing season, *Ech* was responsible for 50-100% of blackleg cases in France and the Netherlands. Also in 2006, the relative incidence of potato diseases caused by *Ech* was high (Wolf *et al*., 2007, Tsror *et al*., 2007). *Ech* has been renamed recently and various strains are now distributed among six different *Dickeya* species.

INTEGRATED PATHOGEN/PEST MANAGEMENT

Potato is planted, harvested, stored and consumed mainly in the vegetative form – the tubers. In this respect the potato crop is different from most major arable crops and imposes special crop protection problems. Management of persistent and recurrent diseases of potato requires the integration of many control measures to achieve an efficient crop protection. The protection of potato started very early before planting.

1. Decisions before potato planting.

Crop rotation. Proper crop rotations enhance soil fertility, help maintain soil structure, increase soil organic matter, and conserve soil moisture, reduce certain pathogen/pest problems - *Rhizoctonia* stem canker, powdery scab, potato cyst nematode, weeds and wireworms (may occur in old grassland or weedy cereal land). Rotations out of potatoes for 3 or more years may be beneficial on some infested sites.

Soil type may affects the incidence of pests and diseases. Heavy, compacted, wet soils favour late blight tuber infection, powdery scab and bacterial soft rots. On the contrary, light freely draining, sandy soils create excellent conditions for common scab and early blight development.

Fertilization. Proper fertilization influences better plants' growth and higher resistance to pathogens. Excessive nitrogen may increase LB on tubers and black scurf potential. Severe attacks of common scab may be encountered on alkaline soils or on soils which have been limed recently. High pH may affect the occurrence of manganese deficiency.

Weeds control. An effective weed management program takes into account the type of weeds present, crop rotation, cultivation, available herbicides, and the competitive ability of the potato crop. Presence of many weeds in potato crops creates high humidity micro-climate favouring LB and rizoctonia development. Weeds are a place of inter-crop survival of pathogens or pest vectors (aphids transmitted potato viruses).

Choice of cultivar. Some potato varieties are more resistant to infection of early and late blight, common scab, virus diseases and powdery scab, storage diseases), pests (potato cyst nematodes), external damage and internal bruising, drought), herbicide tolerance than others. In theory cultivar selection can make a contribution to the prevention of disease. In practice the selection of variety is very much determined by market needs.

2. Control activities during planting

Seed health. Pathogens of the most economic importance, caused potato diseases, including bacterial ring rot, blackleg, common scab, late blight, potato viruses, powdery scab, *Rhizoctonia*, root knot nematodes, silver scurf, and wilt diseases, survive mainly on infected tubers, also in seed tubers. Stem cutting and micropropagation techniques have been developed to obtain pest-free potato plants for propagation and production of certified seed tubers.

Acceleration of plant sprouting in potato crop. *Rhizoctonia* stem canker can be reduced by practices that favor rapid emergence, such as pre-sprouting of seed tubers before planting, planting tubers at a relatively shallow depth, and avoiding early planting dates when soil temperatures are cool.

Seed and/or soil treatments. Control of black scurf or some pests (aphids, Colorado potato beatle and wireworms) by chemical treatment of the soil or seed tubers. Soil treatment may be necessary in case of severe soil infestation with *Rhizoctonia*. Seed treatment limits also silver scurf and common scab.

Good formation of ridges. Tuber infections can occur when spores produced on the plant are washed down the stems or through the soil. Access to the tubers is hindered by a good firm soil coverage with as few cracks as possible.

3. Control of agrophages during growing season

Eliminate of pathogen/pest resources

Potato dump hygiene**.** Growth of potatoes on potato dumps acts as a very significant source of LB, viruses or bacterial diseases. Zero tolerance to any green growth on the pile should be practiced.

Control of volunteer potatoes. Ground-keepers, that is potato tubers left in the ground at harvest can act as a source of LB and virus diseases. Warmer winters in the recent years permit volunteers also assist the persistence of some bacterial and fungal diseases such as blackleg and gangrene.

Negative selection - removing of plants infected with blackleg and stem canker from potato crops, which may be sources of the diseases spread.

Irrigation. Availability of soil water is a major factor that determines yield and quality of the potato crop. Too little water reduces yields but increases severity of common scab or *Verticillium* wilt. Maintaining high soil moisture (80–90% of available water storage) during tuber initiation and the 6 to 8 weeks that follow reduces the severity of common scab and usually controls the disease adequately. Over irrigation may reduce yields and quality, cause several disease problems in the field (powdery scab, black leg), in storage (soft rot), or leach nutrients from the root zone.

Pest and disease chemical control. Main task of chemicals' applications is protection of assimilation surface of potato plants against pathogens (mainly late and early blight, blackleg) and pest (larves of Colorado potato beetle) destruction. In the majority of European countries with high potato production there is conducted a very intensive chemical protection of potato crops against potato LB. Preventive applications of fungicides are advised when environmental conditions are favorable for the disease and continue fungicide applications at 7- to 10-day intervals as conditions require. Shorter intervals may be needed under cool, rainy conditions.

Forecasting and monitoring. Many attempts have been made to forecast blight or provide systems that support the spray decision and replace routine prophylactic spraying. All forecasts are based on determining the probability of weather conditions (temperature, humidity) that will favour diseases and assume the existence of primary blight infection source. Changes in population of *Ph. infestans* influence the earlier LB appears in potato crop. The LB monitoring system on the potato crops aims at the general warning about early attacks of late blight, the investigations of consequence of a possible change in epidemiology of pathogen, analysis of the causes of very early establishment of primary attacks for improvement of existing forecasting system reliability. (Kapsa, Hansen 2004). Currently late blight is more dangerous to potato production than in the past and its control is more difficult.

Decision support systems (DSSs) used in control of potato LB or EB in potato crop protection help to determine an accurate date of first treatment and the following applications – only when necessary. This allows decreasing the number of fungicide treatments that plays a significant role in cost-effectiveness of chemical protection and also in environment protection. Programs of fungicide sprays should commence much earlier before blight is established in the field. Product selection can be steered by crop growth stage.

Haulm destruction can be used to reduce the risk of diseases (tuber late blight, black scurf) by removing the source of infections, preventing contact between tubers and infected foliage at harvesting and additionally decreasing the number of mechanical damages. The reduction in mechanical damage is particularly important to contain the risk of infection with pathogens in store (gangrene, dry rot), but this risk is increased if the interval between the haulm destruction and harvesting the crop is excessive.

4. Harvesting

Preventing bruising and mechanical damages is one of the most important considerations in a well-managed harvest operation. Blackspot and shatter bruise can seriously affect marketable yield if precautions are not taken to reduce them. Several factors are important in controlling bruising: soil moisture, soil temperature and equipment operation.

5. Potato storage management

A large part of the potato crops is stored for fresh market or processing during winter and spring. All seed tubers are stored.

Potato storage facilities can vary but have controls for temperature, humidity, and ventilation. Ventilation is essential during storage. To reduce the risk of rot developing and spreading during the storage season, wet or rotting tubers should be removed from the incoming conveyors when filling a storage unit. The storage period consists of three phases: curing, holding, and warming.

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DEVELOPMENTS IN POTATO STORAGE IN GREAT BRITAIN

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Introduction

Around 6 million tonnes of potatoes are grown in Great Britain annually, with production concentrated in the eastern part of England, the west Midlands and south east Scotland. This overall level of production has been broadly static over the past 40 years but masks almost a doubling of yield per hectare in that time.

The size of production unit has changed much more dramatically, with a four fold increase in the average number of hectares grown per producer in the last decade (Figure 1).

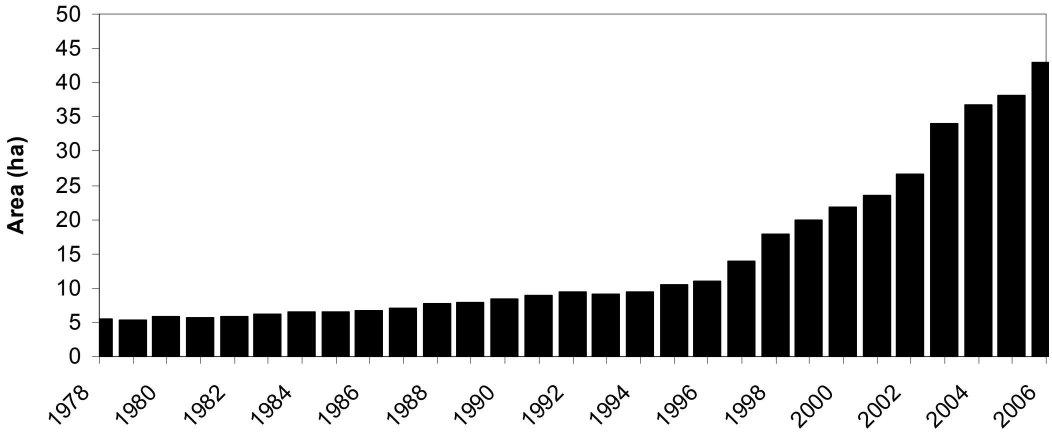

Fig 1: Average size of potato production unit in Great Britain, 1978-2006 (Potato Council data)

This reflects a declining income per hectare of potatoes, in real terms, which means enterprises have had to become larger, more specialist and much more market-focused in the last ten years.

Storage for premium markets

This change in approach has been crucial to success and no less so in relation to storage. Indeed, many of our crops spend longer in the store than they do in the ground. In Great Britain, approximately 3.5 million tonnes of potatoes each year are stored. About half of these are used for the fresh market; the remainder go to a range of different processing and food service markets, which include the major multi-national processors for French fries and crisps whilst about 400, 000 tonnes go to fish and chip shops.

Storage in GB has developed significantly since the 1960s, when the challenge was to be able to keep crops in bulk within buildings without rotting, to the present day when managers look to fine tune the quality of potatoes they deliver from storage to meet the very specific demands of today's markets.

It is this delivery to the quality specification of each market which must be the focus of every potato store manager. To store potatoes successfully, it is essential that this can be done in a timely, consistent and cost-effective way with, ideally, a degree of predictability too.

Fresh markets

The typical British potato store, supplying potatoes for pre-packing and subsequent sale in supermarkets, has a capacity of perhaps 1000 tonnes held in boxes. The store environment will be refrigerated. Crops will be loaded into store in September or early October, ideally within 4-7 days, and immediately cooled down to a holding temperature below 4°C, where they will be held for up to 11 months. During this time they may be treated with ethylene or CIPC to control sprouting or held as low as 2°C to avoid the need for chemical control.

Blemish control is crucial to achieving the best possible returns as skin finish is the primary qualitative factor for these markets. This means it is necessary to prevent the development of diseases like black dot *(Colletotrichum coccodes)*, silver scurf *(Helminthosprium solani)* and skin spot *(Polyscytalum pustulans)* in store.

Black dot has been a major issue in recent years and, at Sutton Bridge Experimental Unit (SBEU), work is being done to look at how the disease can best be controlled, as part of a major project funded by the Potato Council. This research has established that a primary factor affecting black dot risk is the length of time the crop is in the ground. By measuring what has been termed *crop duration*, the time from 50% emergence of the crop until harvest, it has been possible to closely correlate subsequent development of the disease in the field and store. It is therefore likely to be necessary to limit crop duration, at the expense of yield, to ensure that a high quality skin finish is achieved in order to maximise returns. Within the store, black dot control is also compromised if the temperature is not reduced rapidly straight after loading (Figure 2).

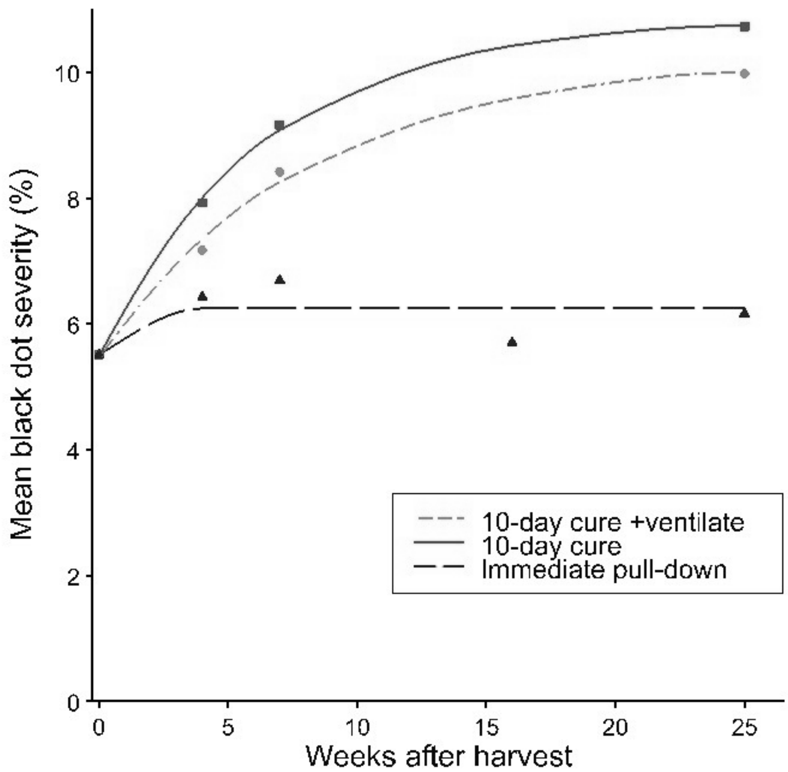

Fig 2: Effect of immediate pull down (rapid temperature reduction) on black dot levels after storage

Silver scurf remains a problem for the fresh market, especially in stores where control is not good enough to prevent temperature gradients within the crop. In these circumstances, at low temperature and high humidity, there is a significant risk of condensation and, wherever there is free moisture in a store, it then only takes a matter of a few hours for silver scurf to become established (Hardy *et al*, 1997), which will compromise the crop's marketability. Skin spot reacts in a similar way (Hilton *et al.,* 2002), albeit with a greater need for curing in susceptible varieties to prevent the disease becoming established on scuffed or damaged skin.

There are currently some conflicting drivers affecting the way in which potatoes are stored for the fresh market, especially in regard to holding temperature. The need to control skin blemishes and to minimise chemical residues on the crop for sale, has fuelled the trend towards lower storage temperatures. However, working in the opposite direction, is the need for lower energy costs (i.e less use of refrigeration) and better taste and texture characteristics associated with warmer storage (e.g lower sugar content). Another factor which may be of relevance is removal of carbon dioxide (CO_2). An initial survey of fresh market storage, undertaken by SBEU in 2007/08, has suggested that some well insulated and sealed, refrigerated stores may need additional venting with fresh air to prevent CO_2 from accumulating to levels in excess of 1%.

Processing markets

The demands of processors are considerably different from those of the fresh sector, and this is reflected in the types of storage used to supply these markets. Typically, storage is in bulk piles, ventilated - at least in part - with ambient air. Crops are grown out to maturity and optimal yield and, when they are loaded into store, temperatures are reduced slowly allowing plenty of time for wound healing. Holding temperatures range from 6 to 13°C, depending on variety, crop sugar status and storage duration. Sprout suppression is achieved using CIPC whilst store management regimes ensure that there is regular, usually automated, flushing of the store with fresh air (perhaps 2-4 times per day for 5-10 minutes) to optimise processing quality (fry colour).

The thermal fogging process used for the application of CIPC has been an area of research at SBEU in recent years in work undertaken in collaboration with the University of Glasgow. By-products from the process have been shown to have a detrimental effect on fry colour and work was undertaken to produce a cleaner fog by fitting a catalytic converter to the exhaust output of a fogging machine. This produced an enhancement of fry colour over and above the effect of ventilating the potato store with fresh air after 8 hours following CIPC application, compared with ventilation after 24 hours (Figure 3).

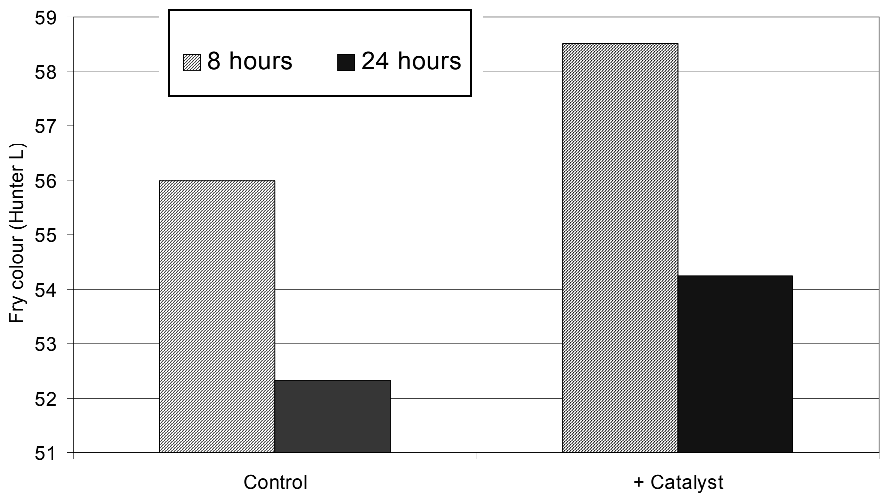

Fig 3: Effect on fry colour of adding a catalytic converter to a CIPC fogger

Other research on CIPC at Sutton Bridge, again in collaboration with Glasgow University, has been focusing on improving application and minimising residues. A three year trial in five commercial stores, which has looked at the use of slow speed ventilation to assist in the application of CIPC to bulk stores, is nearing completion. The application method is a system which has been adapted from that employed in North America and which, on the basis of results to date, looks likely to be adopted in Great Britain. Slow speed fan control is achieved by connecting a variable frequency drive (or inverter) to the fans and, after balancing the delivery of the lower air volume along the length of the store, fans are run during the application of the chemical fog to provide a more uniform delivery of sprout suppressant to the potatoes. The fog is also re-circulated (Figure 4) so that any chemical fog which finds its way out of the top of the pile into the headspace of the store is returned to the crop. By making the application of CIPC more efficient, it has proved possible in trials to get more uniform sprout control across a store and to use fewer applications per season. This is a key concern for store managers in GB at present, as new, lower limits have been imposed on the amounts of CIPC which can be used on any one crop during the season.

Within the processing sector, there are also a conflicting set of influences which have to be taken into consideration when deciding upon a store management strategy. The demand for good fry colours, for those markets where this is a key attribute, has seen a small rise in the typical storage temperature over the last 10 years. However, the new limits on chemical use, are likely to have the opposite effect or, more probably, hasten the move to new, more cold temperature tolerant, varieties. Other key factors are the needs to control energy costs and, of course, to minimise weight loss and disease development in storage.

Store management and control

The general trend for storage in Great Britain, especially with the consolidation of the industry in the last ten years, has been for the major players in the industry to move to better quality stores with more environmental control. For example, this has meant that a higher proportion of the crop is now stored in buildings with refrigeration.

The regimes imposed have also been adapted to take a more specific approach which is based around management of risk. By monitoring quality more closely than ever before, it is possible to identify poorer quality crops and to manage these accordingly, perhaps at a higher temperature or for a shorter storage duration, to improve the consistency of outturns and financial payback from more marginal crops.

This risk-based approach to store management is also the underlying principle of the Assistore™ decision support software which has been developed at SBEU, in association with the Central Science Laboratory (Cunnington *et al*, 2007). This provides advice to store managers which is driven by inputs from them on crop quality at store loading and at intervals throughout the storage period.

The move to more computer-based decision and recording systems is a general trend in storage control in GB. The development of increasingly sophisticated software has allowed a closer level of automated control than has been previously available. However, whilst such systems allow almost infinite variability in the way a store is controlled, it is essential that the consequences of such control are understood. To this end, work to assess the impact of variable frequency drives, which are increasing in popularity in stores, is on-going at SBEU. It is also important that any software is developed with the user clearly in mind. This means ensuring that programs have good, intuitive user-interfaces which make operation of the system straightforward and meaningful to the operator. Some of the better quality commercial systems available still fall down in this respect.

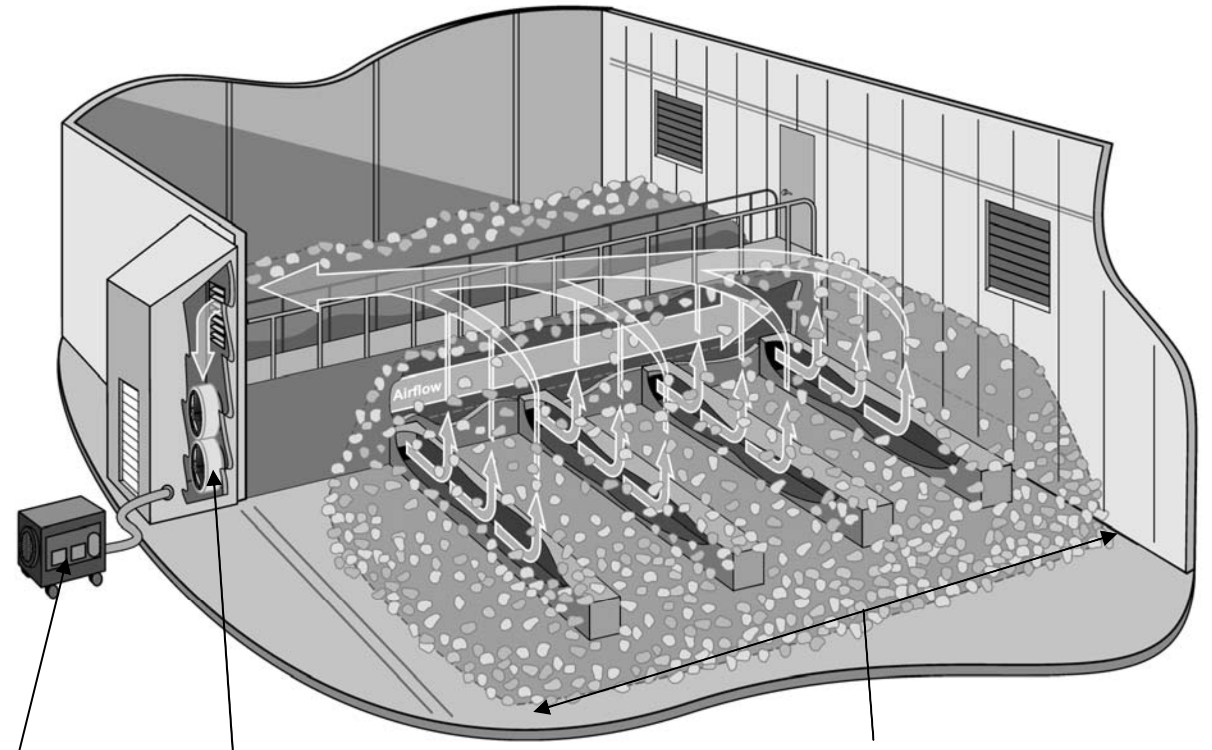

Figure 4. System of application of CIPC to bulk stored potatoes, using speed-controlled fans

Future developments

One effect of increasingly sophisticated storage is that the cost of the process is increased. Ultimately, this has to be recouped through the sale of a high quality potato crop but, with increasing market pressures, every step has to be taken to manage costs.

The rise in popularity of variable frequency drives, whilst beneficial for CIPC application and closer store control, is primarily a consequence of their ability to lower energy consumption in stores. This type of initiative will be increasingly important and other systems which lower energy consumption and enhance store efficiency are under investigation.

The wider use of humidification is a possible area for development, providing stores can be brought up to a standard where condensation risk is minimised. Humidifiers not only offer scope for weight loss reduction but also spore removal (Stroud, 2007) and adiabatic cooling, which allows ambient air to be used for ventilation at warmer temperatures.

The impact of climate change on potato storage in GB could not only lead to a need for better physical resources, such as insulation for stores, but also have a wider impact on, for example, the range of pathogens which have to be managed. There is already evidence of changing risks as a result of shifts in the populations of key storage pathogens such as dry rot (Peters *et al*, 2008), whilst the problems of brown rot and ring rot which are not currently established in Great Briatin, may become more important. The development of quantitative PCR techniques for the assessment of disease is a key technique which will enable better quality diagnosis of storage diseases to be achieved. For example, current research at SBEU, again with Central Science Laboratory, is developing a diagnostic test for skin spot *(Polyscytalum pustulans)* which can be used to detect disease risk on the basis of latent (symptom-free) infections at harvest. Other disease diagnostic tools such as electronic ‘noses’ which sense volatiles given off by disease potatoes, first trialled in Potato Council research in the

1990s (de Lacy Costello *et al*, 1999), warrant further investigation.

Perhaps the most significant need for potato storage in the future is to reduce the dependence, especially for processing, on sprout suppressant chemicals. Minimising residues of the existing products is a first step; removal of the active ingredient from the tuber surface is a second which is currently being investigated by the teams at SBEU and Glasgow University in relation to CIPC.

The introduction of ethylene as a sprout suppressant for the fresh market was a major step forward, because of its 'residue-free' status, but there is a need for more research to understand the underlying effects of such treatments and their potential for use for the processing market (Daniels Lake, 2005). A study in this area is due to begin at Cranfield University, working in collaboration with SBEU, in autumn 2008. However, other options such as dimethyl-naphthalene, used in the USA, and carvone, available in parts of mainland Europe, have not become available in Britain to date. Without the availability of new molecules, therefore, it may be necessary to return to the plant breeders to secure cost-effective and sustainable varieties which offer a chemical-free solution to the problem long term.

In conclusion, despite very significant advances in storage capability and technology over the past 40-50 years, many technical challenges remain to reduce losses, manage costs and consistently deliver quality potatoes from storage in a sustainable way.

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POTATO PROCESSING FOR THE CONSUMER: DEVELOPMENTS AND FUTURE CHALLENGES

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Abstract

In not much longer than half a century industrial processing of ware potatoes into consumer products has developed into a major activity in the potato world. Yearly about 26 million tons of potatoes are converted into consumer products (starch excluded) in the EU and North America. In these parts of the world 20-50% of the daily potato consumption is in a processed form such as French fries and potato chips. Outside the Western world, in Central and Eastern Europe (CEE), Latin America, Asia and Africa the consumption of potato products is rising but still at a much lower level.

Although potato processing is highly industrialized, technologically advanced and very market driven, the quality of its products and the economical success of this industry severely depend on the available potato raw materials. This means that the availability of suitable cultivars, potato yield and quality in its various aspects during the growing season, good post harvest (storage) performance are of utmost importance for the potato processing industry.

In the first part of this paper the developments in potato processing will be described with emphasis on the recent years: the growth of the processing industry into a few global players, the scale enlargement, the consumer trends and market developments, the stabilization of the consumption in the EU and North America and the growth in CEE and Asia, the product diversification and innovation, the changing use of potato raw materials and new cultivars.

The future challenges for potato processing will be subject of the second part. Driven by challenges in market, process and new product development (health?), the focus will be on challenges in potato development for the French fry and related industry: breeding of new cultivars for processing and new consumer products, more sustainable cultivars, genetical modification of potatoes, genomics, organic potato growing for processing, low acrylamide etc.

POTATO BREEDING STRATEGY AND SEED PRODUCTION SYSTEM DEVELOPMENT IN RUSSIA

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Introduction

The Russian Federation is one of the largest potato producers in the world after China. More than 10% of the world potato production is planted in Russia. Potato is one of the main foodstuff products in the country. In 2007 the total planted area of potato field in Russia was more than 2,9 million hectares and the gross yield was 37,5 million tones. In this paper the authors have represented the information regarding potato breeding strategy and seed production system development in Russia.

Important breeding objectives

All-Russian Potato Research Institute began a breeding program, intended to obtain high yielding varieties for different uses and production areas with the heightened resistance to different diseases and pests, high starch content, early ripeness and suitability for the use in the potato processing industry. This program included a wide range of tasks, such as a search and use of new genetic sources, analysis of inheritance of the above-mentioned properties, interspecies hybridization, comparative study of backcrossing variants, and the breeding of new cultivars.

The most important task of the creation of new cultivars is the combination of economically important features with the resistance to different viruses, *P. infestans*, *Alternaria solani*, potato cyst nematode, and stress factors. Main results of this long-term program are the following.

Breeding of cultivars, resistant to viruses

The most harmful potato virus in the Central Russia, where the climate is rather continental, is potato virus Y (PVY), which causes potato degeneration, especially in the case of mixed PVY-PVX infections. Thus, the main objective of our study was to find and isolate sources of resistance to PVX and PVY.

The resistance to PVY^0 was registered for two genotypes of diploid *S. chacoense* species (2n = 24), which demonstrated the presence of a dominant gene after the splitting (R_{ychc}).
We found and isolated also three sources of resistance to PVY in the plants, obtained by the self-fertilization of several backcrosses, carrying R_{Ysto} gene (F_2B_n). The seeds were granted by Dr N.W. Simmonds (England).
Three five-species hybrids, immune to PVY, PVX, and PVS and resistant to PLRV, were obtained from Dr Sh. Sharvari (Hungary). The cv. Fanal was used as an additional source of resistance to PVY, containing R_{Ysto} gene. The cv. Safir, containing R_{xacl} gene, was used as the source of resistance to PVX.

The total number of cultivars, bred on the basis of these sources, was 38, and 27 of them have been included into the Russian State Register. The evaluation of backcross generations allowed us to obtain new data on the breeding value of the sources. The progeny of Hungarian hybrids and *S. chacoense* was resistant to early blight and the hot and droughty weather. Several lines, originating from S.chacoense, were relatively resistant to Colorado beetle (cvs. Peresvet and Nikulinskiy).

To date we bred several cultivars, carrying resistance genes from different sources used: Nakra (chc-sto-dms), Veteran (chc-sto-dms), Russkiy Suvenir (chc-sto-dms), Slava Bryanschiny (chc-vrn-sto), etc. The cultivars and hybrids we obtained from different PVY-resistant sources are widely used now for different breeding purposes, increasing the genetic diversity and heterozygosity of the breeding material.

Breeding of cultivars with an elevated field resistant to P.infestans

The field resistance is controlled by additively acting polygenes, determining the transgressive progeny splitting and the existence of the intermediate stage for the inheritance of this property (Yashina, 1968).

The type of inheritance determined the basic breeding methods we used, i.e. saturating crossings with resistant partners and the selection of transgressive recombinants (TR hybrids), combining a high field resistance with the complex of economically valuable characteristics. To identify TR hybrids, we used an artificial inoculation of detached leaflets with the high-virulent *P. infestans* race (1.2.3.4.5.6.7.8.9.10 XYZ), annually obtained from the All-Russian Research Institute of Phytopathology. The infection load was 25 conidia per microscopic field (120×).

The efficiency of saturating crossings was experimentally tested during the back crossing of PVY-resistant hybrids. Basing on the non-resistant F_1 hybrid (*S. chacoense* 55 d × Agra), we carried out three back crossings and bred new middle-late cultivars (Nikulinskiy and Bryanskiy nadezhniy) with the high field resistance of tubers and leaves. Both cultivars were PVY-resistant. In the back crossings we used cultivars with average (Kameras, Dekama) and high (Mavka, Zarevo) field resistance.

The similar results were obtained during the breeding of cv. Nakra. After the back crossing of the non-resistant three-species hybrid (*S. vernei* × *S. chacoense*) × Anoka with two resistant late-ripening cultivars (Bison, Zarevo), we obtained a mid-ripening cultivar with a high field resistance to *P. infestans*. These data show that after 2-3 saturating back crossings the further back crossing causes an accumulation of polygenes and an improvement of the field resistance.

Now, when we breed new LB-resistant cultivars, we pay special attention to the selection of recombinant forms, or TR hybrids.Basing on TR-hybrids, we created parental lines, combining a high field resistance to *P. infestans* with a high productivity and other valuable characteristics (in 2006 our collection of TR hybrids contained 92 lines).
Hybrids, obtained by the crossings between the mid-ripening cv. Lugovskoy and several middle-late cultivars (Belousovskiy, Zarevo, Nikulinskiy, Peterburgskiy), demonstrated a high field resistance to *P. infestans*.

We use TR hybrids with a high field resistance to P. infestans and other valuable characteristics as parental lines and, at the same time, evaluate them according to the breeding scheme.

Breeding of Potato Cyst Nematode (PCN) resistant varieties

The most widespread pathotype of Globodera rostochiensis in Russia is R0-1, so the breeding material is evaluated concerning this pathotype. In 1977 the first sources of resistance to this pathogenic organism represent CPC cultivars, especially German ones: Hydra (three new cultivars - Bezhitskiy, Zhukovskiy ranniy, and Rossiyanka - were originated from this cultivar), Gelda (Lukyanovskiy and Zavorovskiy), and Kardia (Aspiya, Solnechniy). The use of each of cvs. Elvira, Kulpe, and Oktavia allowed breeders to obtain one new cultivar. Recently we bred new nematode-resistant cultivars, basing on our own cvs. Malinovka and Krepysh.

Basing on the S.chacoense 58d, we bred cv. Bryanskiy delicates, Nakra, Slava Bryanschiny, resistant to R0-1.

Since 2005 we use a special collection of cultivars and hybrids, resistant to R0-1 pathotype and including 36 forms of different origin; 18 samples from this collection are original hybrids, combining the R0-1 resistance with the early and late blight resistance and also the resistance to some viruses.

We use new PCN-resistant cultivars, bred in Russia (Pushkinets, Nayada) and Belarus (Albatros, Atlant, Rosinka, etc.), as the starting material; in our crossings we also use some foreign cultivars, such as Ausonia, Sante, Aroza, Maestro, etc.

To increase the efficiency of the laboratory testing of the resistance to R0-1 pathotype, we improved the assessment methods, that, in turn, improved the accuracy of the resistance assessment, carried out on the basic stages of the breeding process (Simakov et al., 2006).

Breeding of cultivars with a high starch content

In Russia a high starch content is traditionally associated with the good taste of potato tubers and their mealiness, so the optimal starch content for the table cultivars is considered to be equal to 18-20%. However, adverse weather conditions and a short vegetation season, typical for the central Russia, often decrease the value of this parameter by 2-3%.

The way of inheritance of the starch content and the small number of loci, responsible for this property (Yashina, 1982), allow us to use several simple methods for the improvement of this characteristic. These methods include the use of parental lines with the heightened starch content and the selection of positive transgressions in the progeny. However, a negative correlation between a high starch content and a high yield complicated such breeding work. This fact explains the low frequency of recombinant hybrids, combining a high starch content with the high yield, in the most of populations.

Therefore, the preliminary estimation of hybrid populations concerning the frequency of recombinant genotypes can be very important for a successful breeding.

Most of cultivars with the high starch content are late-ripening, so one of the important directions of our work is a breeding of early and mid-early cultivars with the heightened starch content. Today, basing on the radiation selection and mutagenesis, we created a new starting material, characterizing by the combination of the mentioned properties (Simakov et al., 1991). We also obtained first hybrids with potential valuable characteristics; their testing will allow us to determine the most effective breeding methods.

Breeding of new cultivars for potato processing industry

The breeding of potato cultivars for potato processing industry is a relatively new direction of our breeding work. The first studies were carried out in 1980-1983, when we estimated the starting material and analyzed the inheritance of this property.

The analysis hybrid populations allowed us to specify the period of evaluation of the breeding material for its suitability to processing in different periods of storage. We also specified methods of selection of parental forms and established that the optimal breeding should include one or more “suitable” parental genotypes in every crossing.

According to the results of estimation of 36 hybrid populations, we selected 20 hybrids, highly suitable for processing over the whole period of storage. These hybrids are excelled by the complex of other valuable characteristics and are used as the starting material in this field of selection.

Improvement of breeding methods

The successful breeding is determined not only by the expansion of the genetical base of a breeding material, but also by the use of reliable methods of evaluation and selection of the valuable genotypes. The testing of the adaptability of breeding material to uncontrolled environmental factors and different pathogens is especially important.

To study the influence of the selection background, we developed the program of parallel evaluation of identical hybrid populations in different soil and climatic zones of Russia. To evaluate the breeding, our breeding center formed 3-4 identical sets for each studied population and then sent them to different breeding institutions of European part of Russia, Siberia and Far East. All these institutions tested the populations according to the common breeding scheme.

The efficiency of selection was estimated by the number of cultivars, registered in the State Register. Such trials showed that new cultivars are selected from different populations in different ecological regions. Only four case of the sibling selection was registered. Due to such parallel trials in different regions, the share of cultivars, selected from the ours populations, significantly increased (Simakov et al., 2003). During the last decade (1998-2007) our institute bred 21 new cultivar, and other 10 cultivars were selected from the same populations in a collaboration with other breeders; the total number of created original cultivars, suitable for different soil and climatic conditions, is 52. The efficiency of selection from the estimated populations increased more than twice.

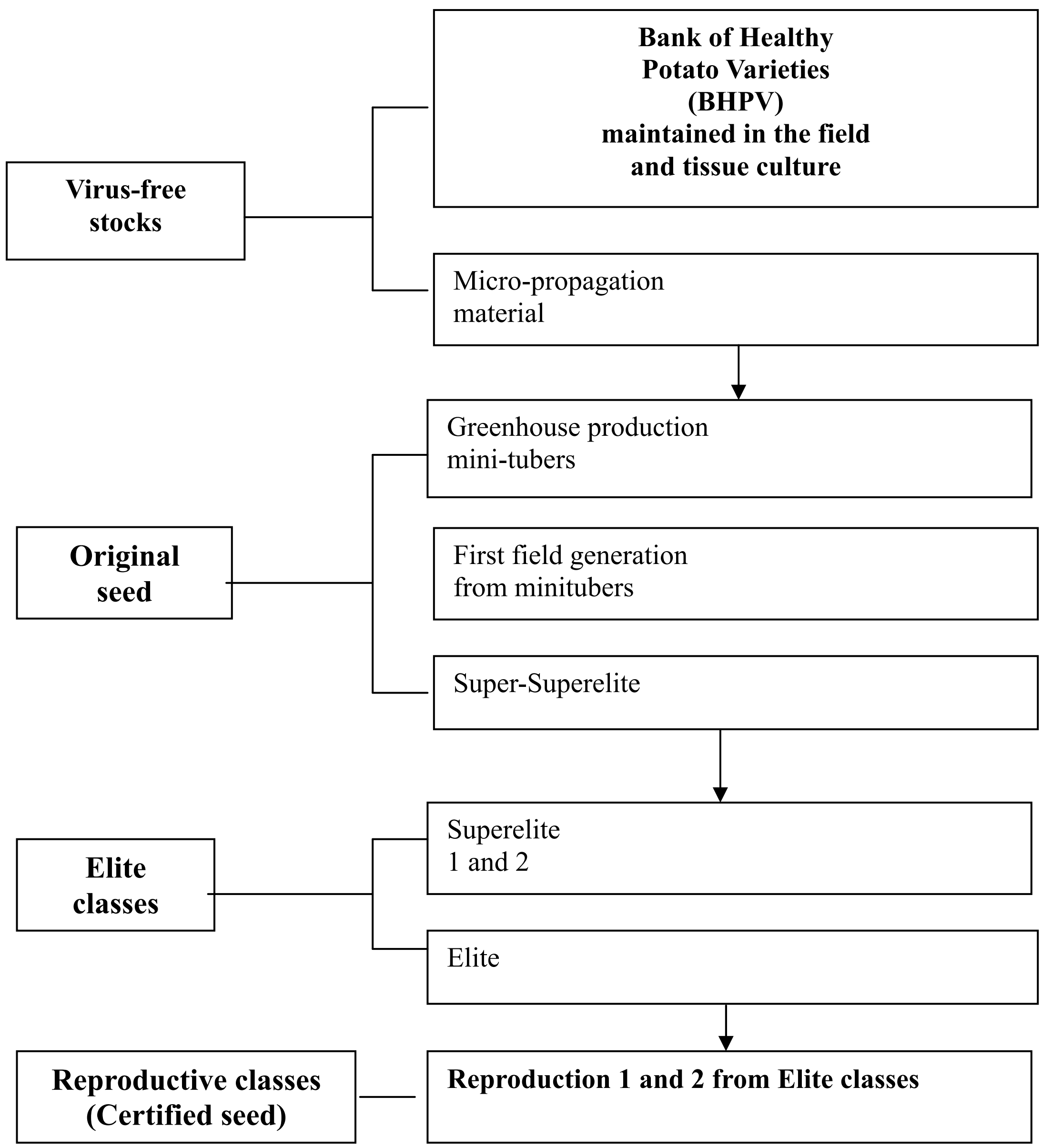
As a whole, the results of performed studies confirm the use of various selection backgrounds to be more promising, than the work with the single background and in the single ecological region. A multiple use of identical hybrid populations in the selection increases the breeding efficiency and significantly decreases financial expenditures for the breeding of the new cultivar.

In recent years we use some new molecular and genetic methods, including PCR analysis, for the genotyping of different potato cultivars, investigation of the variability of meristematic lines, identification of mutations, detection of viruses and viroids, and investigation of the breeding material using DNA markers of genes, determining the resistance to different pathogens. We also started a program of creation of transgenic potato cultivars.

Seed potato system development

According to the "Breeder Achievements Law" (1993) and "Federal Seed Law" (1997) in Russia seed potato is divided in the following groups (categories): virus-free stocks and micro-propagation material; original seed material; elite seed classes; reproductive seeds (Figure 1).

Figure 1. Seed potato classification in Russia (Anisimov,.Uskov,Varitsev and Yurlova, 2007).



As it is well known the possibility of viral infection of potato plants and virus transmission into tubers are determined by place and conditions of growing, level of infection pressure (amount of insect vectors and infection sources), resistance of cultivars and other factors.

In Russia the production of original seed material and elite classes on the base of traditional system was created in different regions where the level of infection is minimum. Taking into consideration a wide diversity of agro-climatic conditions, infection loading level and number of insect vectors, we can mark out the three main groups of Russian regions (Anisimov, 2005):

1. The Northern, North-western and North-eastern regions have most favourable conditions for cultivation of quality seed potato. For example, Leningradskiy region is situated on the Baltic Sea coast. It has cool summer weather and relatively low background of insect vectors. These conditions conduct to minimum the expansion of most heavy virus infections. Vegetative period in this region is very short - from end of May till middle of September (100-110 days), however this region has a very long light day (till 23 h). It creates very good conditions for fast potato growth and maturity. These agro-climatic conditions make this region favourable to cultivate high quality seed potato.
2. The Middle part of Russia (Central, Central black-earth and Volgovyatka regions, Ural, Siberia, Far East) has a wide diversity in the soil content and fertility, rainfalls, sum of active temperatures, frost-free period etc. However these regions can be characterized like regions with moderate infection pressure and can be considered favourable areas to organize own quality seed potato production.
3. The South and South-East regions have less favourable conditions to cultivate potato and organize own quality seed potato production. These regions have hot and dry climate during vegetative period and high infection pressure level (excepting of highlands in the North Caucasus area). Therefore the rates of accumulation viral infections with each following field generation are much higher than in Central regions. A high rate of spread of infection decreases potato production. Quality of seed potato becomes much worse after 2-3 vegetative periods (or only after 1 vegetative period at the susceptible varieties).

For the most optimal prophylactic effect to be obtained in seed potato cultivation the following requirements are met:

- Guarantee of a complete absence of quarantine pathogens (potato wart disease - *Synchitrium endobioticum***,** golden nematode of potato – *Globodera rostochiensis,* brown bacterial rot - *Ralstonia solanacearum;*
- Absence of soil viruses (TRV and PMTV) and their vectors *(Trichodoris spp.,Spongospora subterranea;*
- Minimal risk of wide spread of phytopathogens in the field conditions (absence of PRLV, PVY, PVM virus pestholes and their vectors for a 2 km around);
- Minimal likelihood of bacteria spread (blackleg - *Pectobacterium atrosepticum,* ring rot – *Clavibacter michiganesis).*

Quality control and Seed Certification development

The main objectives of quality control are:

- Varietal purity and diseases control in the field: it includes 3-time field assessment during growing period (Elite classes – 2 assessments and reproductive classes – 1 assessment);
- Lot inspections are realized on the basis of visual observations means of tuber analyses.
- All original seed material produced from micro-plants and mini-tubers are tested by ELISA (post harvest control);

Potato viruses are tested in laboratory by ELISA and other laboratory methods. The scheme of laboratory control for different generations (seed classes) is shown in the Table1.

Table 1. Norms and methods of virus detection for different generations (seed classes)

Generation (class)	Testing norms	Methods
In vitro material	100% of plants	Elisa-test, NASH, PCR etc
Mini-tubers in greenhouse	100% of plants	Elisa-test
1-st field generation	200 plants per plot	Elisa-test
Super-super-elite	200 tubers (postharvest test)	Elisa-test
Super-elite		Visual symptoms assessment
Elite and Reproductive classes		Visual symptoms assessment

According to the data of State Seed Inspection of Russian Federation for potato planting in 2007 crop season more than 300000 tones of seed potatoes in a wide range of varieties were certified. It was more than 150 multiplied varieties including Russian varieties Nevsky (82000 tones), Udacha (29000 tones), Elizaveta (1020 tones), Zhukovsky ranny (4200 tones), Volzshanin (3500 tones) and Foreign varieties Rosara (27000 tones), Red Skarlet (22500 tones), Romano (22300 tones), Saturna (12200 tones), Karatop (10500 tones).

Current trends in the Russian seed potato production

For quality improvement of seed potato in Russia the new project was started in 2004. A very important part of this project is the Bank of Healthy Potato Varieties (BHPV). This Bank is located in the Northern area (Archangelsk region) and maintained under the cleanest phytosanitary conditions (Simakov and Anisimov 2006).

Results of quality control of original seed material produced by traditional and new system are shown in the table 2.

Table 2. Comparative results of laboratory testing of original seed material (Moscow region, 2007)

Varieties	Viruses, %		
	Total	PVX+PVS+PVM	PVY+PLRV
	Original seeds, produced by traditional system		
Zhukovsky ranny	2,5	2,0	0,5
Udacha	2,5	2,0	0,5
Nevsky	4,0	4,0	0

	Original seeds, produced on the base of the BHPV		
Zhukovsky ranny	0,5	0,5	0
Udacha	0	0	0
Nevsky	2,0	2,0	0

To ensure a transfer to the adaptation of a uniform seed potato certification scheme in Russia, the following activities are planned:

- Introduction of a compulsory set of requirements for virus control of original and elite seed potato categories, using laboratory test methods on the basis immune-enzyme analysis/ELISA-test and also immunochromatography method on a test-strips (immunostrips) for analysis of field basic plants (clones).
- The measures are necessary to develop a net of regional testing laboratories with modern equipment, technical means and diagnostic kits for a full range of laboratory tests for viruses, bacteria and other phytopathogens which are heavily decreasing the quality of seed potatoes.
- To organize the centers of phytosanitary monitoring in the regions on the base of research organizations with involvement of plant protection service specialists and regional State seed inspectors. Their activities should include the phytosanitary monitoring of movement dynamics of virus transferring insects, definition of their varieties and notification of seed production farms of the dates of mass aphis migration with the purpose of definition of the optimum dates for leafy tops removal on various seed potato plantations of various classes and categories.
- To arrange on the basis of All-Russian Potato Research Institute and Certification Centre "Test Potato Service" the regular short-term trainings, practical trainings and consulting services for the specialists with the purpose to improve the practical skills in recognizing of variety properties and disease symptoms on potato plants and tubers, as well as trainings of specialists in post-harvest control of seed potatoes by means of ELISA.

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ADVANCES IN FUNCTIONAL GENOMICS AND GENETIC MODIFICATION OF POTATO

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The challenges facing potato breeding have actually changed very little over the years with resistance to pests and pathogens remaining high on the agenda together with improvements in storability, reduction in blemishes and novelty and consistency in cooking /processing qualities. The need to expand the range of targets for potato improvement is being driven by requirements for reduced agrochemical usage and by predictions of the effects of changing climates. Thus fertilizer use efficiency and water use efficiency are moving up the political agenda. Not withstanding this, the industry is still faced with some degree of uncertainty with food safety issues such as acrylamide formation. More than ever, the genetic variation present in germplasm collections needs to be harnessed to provide the genes and alleles required

The complex genetics of tetraploid crops such as potato is well documented, together with the fact that it can take up to 15 years to develop a potato cultivar through conventional traditional breeding approaches. More efficient and effective conventional breeding strategies have been developed (e.g. the use of multitrait selection approaches) but most characters relevant for crops show continuous phenotypic variation because they are controlled by multiple genes (quantitative trait loci, QTL) and by environmental factors. It is now possible, through advances in statistical approaches, to assemble maps of molecular markers and locate QTLs affecting quantitative traits in autotetraploid species. However, knowledge of the actual genes and alleles regulating traits of interest remains the panacea for plant breeders as the candidate genes can be deployed in marker assisted selection or used in transgenic approaches for trait improvement.

The revolution that has occurred in our capacity to sequence DNA has led to explosions in genome sequencing initiatives, including those for the potato genome. These include the National Science Foundation (NSF) Potato Genome project (USA), the Canadian Potato Genome Project and an international Potato Genomics Sequencing Consortium (http://www.potatogenome.net) established with the objective of elucidating the complete sequence of the potato genome by 2009. Once the genome projects are completed the research paradigm shifts towards the analysis of the function of the gene networks and systems. However, genome projects normally proceed in parallel with other initiatives aimed at identifying gene function. The term "functional genomics" is a catch all phrase which encompasses several approaches which can be used to identify and ascribe functions to genes. These include:

a) Map based cloning of target genes using markers tightly linked to the gene in question
b) Transposon tagging, activation tagging and reverse genetics
c) Transcriptomics linked to phenotyping to link gene expression with trait development
d) Transgenic approaches, using either stable or transient gene expression systems

The plenary lecture will give examples of functional genomics approaches leading to an improved understanding of mechanisms underpinning potato traits including disease resistance and product quality. The presentation will also provide examples of the use of emerging technologies such as metabolomics to identify potential gene targets, particularly when used within a framework of a comparative analysis of germplasm which is clearly differentiated in term of quality.

Genetic Modification

As state above transgenic approaches are extremely useful for gene functionality testing (experimental use) but for potato should also represent an important component of the breeders' toolkit. However, due to concerns primarily over the safety of GM crops to human health and the environment, uptake of the technology and GM crops has been extremely limited within the EU, despite the establishment of EFSA as an independent risk assessment body and the implementation of new EU Directives and Regulations dealing with food and feed safety, detection/traceability, and releases into the environment. There are currently no GM potatoes grown in the EU and the first one likely to be approved will be for industrial starch production. Even in the USA, the demise of the Monsanto Company's NatureMark unit means it is unlikely that any de regulated GM potatoes remain in the market.

The presentation will provide examples of the range of potential impacts that GM approaches could make. It will also consider the value of so called intragenic or cisgenic approaches to potato genetic engineering. These approaches involve transforming plants with native potato DNA sequences thus, in theory, could also be accomplished by traditional breeding. There is growing evidence that such products would raise fewer concerns with consumers and some argue that the risk assessment and de regulation of such crops should be streamlined.

Whilst functional genomics tools can be used to identify potato improvement targets there is an ongoing debate over the potential value of much broader scale, more unbiased analytical approaches in the risk assessment of GMOs which, through the quantity of data they generate, may help to a) identify effects which could stimulate the need further risk assessment b) reduce the level of uncertainty that unintended effects have occurred. Most of this debate has clearly focused on GM crops but it is already well established that significant natural variation exists within crop gene pools and that this is accentuated by the prevailing environment. Crop management practices are also likely to affect crop composition. Thus, for benchmarking and comparative analysis approaches, the deployment of broader scale, less biased analytical approaches for GM safety assessment should also embrace the issues of sources and extents of variation. Examples of these approaches applied to potato grown under a range of cultural practices will be discussed.

EXPLOITATION OF EXOTIC, CULTIVATED *SOLANUM* GERMPLASM FOR BREEDING AND COMMERCIAL PURPOSES

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Introduction and history of potato

Potato has probably more related wild species than any other crop, since the genus *Solanum* comprises around 2000 species. Among them figure 235 tuber-bearing species (2n=2x=24 to 2n=6x=72) but only seven of them including some hybrids are cultivated (Hawkes 1990).

Two biodiversity centres in central Mexico and the Altiplano between Peru and Bolivia are considered. The disappearance of the terrestrial bridge between Central America and South America during the age of the Pleistocene 30 million years ago might have separated them. According to Hawkes (1967) *Solanum stenotonum* derived from *S. leptophyes* was the first cultivated species *S. andigena* (referred to as *S. tuberosum* ssp. *andigena*), the pre-cursor of tetraploid potatoes, originated from hybridizations between *S. sparsipilum* and *S. stenotonum*. This latter species represents probably also the origin of some other *Solanum* species.

Domestication took place around 10.000 years ago in western Bolivia. (Engel 1964). The possibility to conserve and to store potatoes by processing them to "Chuño" (a type of freeze-dried potato) was one of the key inventions for the domestication success.

The Chiloe Island (Chile) represents a diversity centre for S. andigena and may be also the origin of *S. tuberosum* ssp. *tuberosum*. Some authors believe that in Europe *S. tuberosum* ssp *tuberosum* originated from long-day adaptation of S. andigena (Salaman 1985) while others think that these were directly imported from Chile.

The first written references for potatoes were from F. López de Gomara (1552) and P. de Cieza de León (1553) which name them "turma de tierra (earth tuber)" and mention the local Quetchua name "Papa". Caspar Bahuin was the first to describe potato (S. tuberosum) in 1596, 157 years before Carl von Lenné (1753; Species Plantarum), however, with a slightly different name.

There were several introductions into Europe around the same time. John Hawkins reported loading potatoes in Venezuela on March 28, 1565. Popular legend has long credited Sir Walter Raleigh with first bringing the potato to England, but history suggests Sir Francis Drake as a more likely candidate. In 1586, after battling the Spaniards in the Caribbean. According to Ruiz de Gordoa (1981) potatoes were sent from Cuzco to the Spanish king Felipe II in 1565, but could be introduced even earlier in the Canary Islands (1562).

After the potato was brought to Europe, it gradually spread across Europe. Initially the food was treated by distrust, and was believed to be unhealthy and even un-Christian. This prevented widespread acceptance for a period, although it began to be promoted as an ornamental and medicinal plant. During periods of warfare, the potato proved beneficial. Grain crops could be burned down by marching armies, but potatoes lay underground and were generally unaffected by military foraging. This led to increased use by the peasantry of areas in Germany, which had been particularly badly ravaged during the Thirty Years' War. Agriculturalists in Europe soon found potatoes easier to grow and cultivate than other staple crops, such as wheat and oats; potatoes produce more food energy than any other European crop for the same area of land and require only a shovel for harvesting. The famine caused by Late blight in Ireland (1840s) was a serious drawback of potato in many countries. Today, potatoes grow widely in Europe, especially in North European and eastern countries including Russia and represent national dishes in several countries.

Native Potato species and their properties

While in Europe all modern potato cultivars belong without exception to *S. tuberosum* ssp *tuberosum*, in South America also other tuber-bearing species are cultivated, which are called Native Potato Species (NPS). Among them figure *S. sparsipilum* (2x), *S. stenotomum* (2x). *S. ajanhuiri*

(2x) with two groups Yari and Ajawiri, *S. megistacrolobum* (2x), *S. curtilobum* (5x) and S. *phureja* (2x) and *S. tuberosum* ssp. *andigena*.

In part these NPS are cultivated under harsh environmental conditions were potato cannot compete. For centuries, they have been locally selected by the Andean farmers in order to provide subsistence under the harsh environmental conditions of the Andes. The farmers were able to select and maintain a highly diverse germplasm with excellent organoleptic quality by cultivating native potatoes with different ploidy, maturity, level of resistance to pests, diseases and stresses in the same field. However, until now these valuable resources have not been exploited efficiently due to the geographical isolation (Huaman 1982).

Some old introductions of native potato species exist also in Europe. In Canary Islands the "papa negra" (= *S. chaucha*) is highly appreciated. Despite of its low productivity, the high market prices make them a lucrative crop. Occasionally, we can also find some *S. phureja* materials from Colombia or Ecuador such as the "Criolla" or "Yema de Huevo" which are sold at high prices in European markets.

A broad range of pests and diseases including quarantine pathogens affects the potato crop. Not only *Solanum* wild species, but also cultivated native species possess resistances against different pest and diseases. Specific resistances have been reported against various potato viruses in certain accessions of native potato species. Also resistances against *Streptomyces scabies*, against the nematodes *Globodera rostochiensis*, *G. pallida*, *Meloidogyne incognita and Nacobbus aberrans* and the dangerous oomycete *Phytophthora infestans* have been detected. In fact, further studies on Andean cultivars have shown that some accessions have multiple resistances to pests and/or fungal, bacterial and viral diseases (Huaman 1982).

Native potato species are cultivated at altitudes between 2000 and 4200m above sea level. Frequently they are exposed to severe heat, solar radiation, drought conditions, or severe frosts during the night. They can be found also on poor and acidic soils with increased levels of heavy metals. The group of native potato *S. ajanhuiri* is grown in the harsh conditions of the Bolivian Altiplano and most of the frost tolerant potatoes belong to the group *juzepczukii*, *curtilobum* and *ajanhuiri* (Huanco 1991). Potatoes are known to have favourable nutritional properties. In recent studies high protein contents were found in some native potato accessions (Huaman 1982). Some represented also very good sources of vitamins B6, C, B3, B5; dietary fibre; and the minerals copper, potassium, iron and magnesium. Potatoes also contain a variety of phytonutrients that have antioxidant activity. Among these important health-promoting compounds are carotenoids, flavonoids, caffeic acid and the tuber storage protein patatin, which exhibit activity against free radicals and have antimicrobial, anti-inflammatory and anti-allergic properties. Larger amount of flavonoids can be found in potato. Yellow fleshed diploid accessions from *S. phureja* and *S. stenotonum* have a very high carotenoid content (Lu et al. 2001).

Native potatoes are well known for their organoleptic qualities and processing abilities. Recent studies have shown that native potatoes can exhibit a very high dry matter content (over 25%), leading to reduced oil absorption and therefore produce high quality fries and chips (Bonierbale et al. 2004).

Native potato species and breeding

These properties make NPS interesting objects for potato breeding and some genotypes could be used as progenitors to introduce resistances or quality traits. Introgressions from already cultivated species are more efficient than from wild species for obtaining rapidly the desired varieties.

However also some unfavourable characteristics of certain NPS accessions have to be mentioned.

One mayor drawback is for sure the missing long-day adaptation in most of the genotypes of all species, although a certain degree of variation can be observed.

The lack of dormancy of *Solanum phureja* has always limited the use of these native potatoes which contains very tasty ecotypes. Furthermore, observations have shown that some genotypes are also very sensitive to pests and diseases during storage. Storage ability is an essential trait for subsistence of farmers in the Andes, but also for potato breeding.

While native species contain clones with highly valuable traits, the use of "exotic" uncharacterized *Solanum* species within breeding programmes may potentially imply also some risks for human health. Therefore the properties with respect to certain harmful substances of breeding material have to be assessed properly before using them as crossing partners.

Tuber-bearing Solanum species exhibit wide quantitative and qualitative diversity in glycoalkaloid contents. Some species contain extremely high total glycoalkaloid levels of ill-defined toxic and teratogenic properties (Gregory 1986). Some native frost-resistant cultivars of S. juzepczukii present a very high content of glycoalkaloids, but the Andean farmers have learned how to process these cultivars before making an edible product, the "chuño" (Brown 1999).

Another potential problem is nitrate toxicity. Ingested nitrates can be converted into nitrosamines, which are known carcinogens or can be reduced to nitrites, which decreases the oxygen-carrying capacity of haemoglobin. Nitrate concentrations depend on environmental factors, but exhibit a broad range of natural variation in Solanum species (Gelder et al. 1988).

Traditional cooking methods, such as baking, frying, and roasting, can generate the formation of carcinogenic acryl amide. Public health organizations are concerned about the potential risk of acryl amide to human health (Biederman & Grob 2003). Therefore, it is necessary to evaluate the potential to accumulate harmful substances in native potato species in thorough bio-safety assessments before deciding on their suitability for potato breeding.

Commercial Products derived from NPS

The value of NPS for generating additional income for local farmers through commercial exploitation has been recognised in their countries of origin. NPS are promoted by organisations such as Proinpa (Bolivia), INCOPA/Papa Andina and T´ikapapa (Peru). T'ikapapa is a creative, sustainable partnership between potato-chain stakeholders and small-scale farmers. It represents an example of a new marketing concept that demonstrates that it is possible for Andean farmers to capitalize on the added values of biodiversity and tap into exclusive markets. T'ikapapa-trademarked fresh native potatoes are now being sold through Wong, Peru's largest supermarket chain.

Several other products derived from NPS have been launched also on the market. These include the traditional "chuño" which is now sold pre-packed for making soups or potato purée processed from yellow *S. phureja* ecotypes. Accessions from the *goniocalyx* group have outstanding properties for chips while the excellent flavours, varied shapes, pigmentation patterns, and attractive colours of some other native cultivars resulted in chip products with considerable novelty appeal (Bonierbale et al. 2004).

While in Europe NPS certainly can not compete with traditional *S. tuberosum* based cultivars, but may be useful to introduce certain characteristics through breeding, there is a certain niche market for high value-added speciality products in the delicatessen branch. This requires, however, a certain long-day adaptation to improve yields can be achieved through breeding. One might consider the mentioned products in their countries of origin such as the yellow, tasty potato purée or the "designer" chips. Also a bowl of undersized "baby" potatoes with different skin and flesh colours served with different dips represents an attractive first dish. Several products are already commercialized such as the purple flesh potato variety "Blue Congo". This cultivar is popular in Scandinavia, especially in Finland, where it is served mashed with sour cream. More recently the *S. phureja* derived cultivars "Inca Sun" and "Mayan Gold" were obtained by SCRI. Breeding work was started already in the 1960's. Mayan Gold has a floury texture, excellent taste, golden coloured flesh, is rich in carotenoids and cooks extremely quickly. Initially these cultivars were destinated to the home gardener retail market, but consumers appreciate these new products and their market is growing.

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BREEDING FOR PHYTONUTRIENT ENHANCEMENT IN POTATO

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Tubers of 38 native potato cultivars of different taxonomic groups from South America were analyzed to determine the total anthocyanins, total carotenoids and antioxidant values of several groups of breeding clones and varieties were analyzed. Total anthocyanins and an Hydrophilic Oxygen Radical Absorbance Capacity (H-ORAC) were measured in the acetone fraction of an acetone chloroform phase separation. Total carotenoids and a Lipophilic ORAC (L-ORAC) were measure from the solutes in the chloroform fraction. In a a group of native cultivars of South America, total anthocyanins ranged from zero to 23 mg cyanidin equivalents/100 g fresh weight (FW). Total carotenoid ranged from 38 to 2020 µg zeaxanthin equivalents/100 g FW. The hydrophilic ORAC ranged from 333 to 1408 µM Trolox equivalents /100 g FW. The lipophilic ORAC ranged from 4.7 to 30 nM α-tocopherol equivalents /100 g FW. The cultivars consisted of 23 diploids, seven triploids and eight tetraploids. Total carotenoids were negatively correlated with total anthocyanins. Total anthocyanins were correlated with hydrophilic ORAC. Among clones with less than 2 mg cyanidin equivalents/100 g FW total carotenoids and lipophilic ORAC were correlated, but this was not true for analysis of all 38 clones. Although total anthocyanins or hydrophilic ORAC values reported here were not outside of the ranges found in North American and other breeding materials, total carotenoids and lipophilic ORACs are higher than previously reported, suggest that native cultivars of South America with high levels of total carotenoids and high lipophilic ORAC are a unique germplasm source for introgression of these traits into specific native potato cultivars outside the center of origin.

A breeding effort designed to increase the antioxidant level of potato by means of high concentrations of anthocyanins and/or carotenoids provided selected materials for a second analysis. Total anthocyanins varied between 9.5 and 38 mg cyanidin equivalents per 100 g FW. The hydrophilic fraction ORAC measurements among anthocyanin-rich clones varied between 250 and 1420 umoles Trolox equivalents per 100 g FW. These two variables were significantly correlated, $r = 0.73$, and with significant positive slope in linear regression. Measurement of total carotenoids revealed differing degrees of yellowness that spanned a range of total carotenoids extending from 35 to 795 µg zeaxanthin equivalents per 100 g FW. Dark yellow cultivars had roughly ten times more total carotenoids than white flesh cultivars. The lipophilic fraction ORAC values ranged from 4.6 to 15.3 nmoles α-tocopherol equivalents per 100 g FW. Total carotenoid was correlated with the lipophilic ORAC values, $r = 0.77$, and also had a statistically significant positive regression coefficient. Clones with red and yellow pigments visible in the flesh had anthocyanins and carotenoids in elevated levels and ORAC contributions from both fractions. Although anthocyanins and carotenoids are major contributors to antioxidant activity, other constituents of potato flesh likely play significant roles in total antioxidant values.

High carotenoids potato may have particular value for human health due to the antioxidant properties and the therapeutic value for eye health in patients at risk for macular degeneration. Carotenoid concentrations were determined among the progeny of a cross between two high carotenoid lines derived from diploid *Papa Amarilla* germplasm from South America. The total carotenoid content ranged from 82 to 2686 micrograms zeaxanthin equivalents per 100 g FW. The higher values greatly exceeded the mid-parent value of the cross. A cleaved amplified polymorphic sequence (CAPS) assay was developed to distinguish the alleles of beta-carotene hydroxylase (*bch*) in the two high-carotenoid parents. A *bch* allele (denoted B) common to the high carotenoid parents co-segregated with yellow flesh in the progeny of a white flesh x yellow flesh cross, making *bch* an excellent candidate for the classical *Y* locus, required for yellow tuber flesh. The same allele was also present in all other yellow flesh potato clones tested. Genotype at the *bch* locus explained a portion of the variation of total carotenoid (R^2 = 0.42). Clones homozygous for the B allele (BB) contained, on average, slightly more carotenoid than heterozygous Bb clones, which in turn had much more carotenoid than homozygous bb clones, suggesting a partially dominant gene model. Similarly, bb flesh was significantly less yellow than *Bb* and BB, the latter two being quite close. Total carotenoids varied considerably among progeny in the Bb and BB genotype categories suggesting that variation at one or more additional loci have a significant effect on total carotenoid levels.

Examination of iron contents in advanced potato germplasm and established varieties indicated that there was a of 3-4 fold range from lowest to highest.
The possibility of developing higher iron cultivars in which the iron is highly bioavailable due to the presence of ascorbic acid, also at high levels has important implications. Iron deficiency affects 50 percent of the world's poorest populations. Potato might contribute to the improvement of this problem.

POTATO BREEDING – A CHALLENGE, AS EVER!

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It is a common breeder's claim that plant breeding is as old as the agriculture itself may be. Therefore, the two fundamental questions "Why?" and "How?" they have to answer today are nothing new! From the very beginning, farmers and growers that were the very first plant breeders, have answered very simply: keep the best for the best!

Still, the best has so many different meanings and values around the world that the answers to the "Why" and the "How" have been highly diversified and always challenged!

Until recently, the "Why" and the "How" have been two unrelated questions. The best "Why" has been relatively easy to define: in food and feed crops, traits related to the quantity of product (yield) have been given the priority while in ornamental, major traits have been related to the quality (colour). From the discovery of Mendel's laws of the inheritance of traits, "How" has not been really a question until now: making crosses and screening has been the universal natural approach. I would say that the best answer to "How" has been to hire a qualified breeder who is a hard field worker and who has got rigour and patience alongside expertise and passion.

For the recent decades, the growth of scientific knowledge in agronomy, physiology, genetic, plant protection, statistics and data management has allowed for more rational choices in breeding aims, tools and methodologies. But also it pushed the agriculture, the food industry and the plant breeding activities in the middle of public controversy.

Today, the "Why" and the "How" are no longer unrelated questions. Furthermore, quality and quantity have to be combined. Then the challenges for the best "Why" and for the best "How" are increasingly sophisticated!

Challenge on « Why »

Worldwide, one of major evolutions for the last two decades has been the growing of the potato exchanges between traditional potato countries and the potato development in new regions having no or young tradition of potato.

In EU, one might reasonably think that most of the demands could be satisfied thanks to the 1200 potato varieties that have been currently registered in the EU list. Nevertheless, evolutions in cultivation, storage, processing and distribution have been continuously challenging breeding research to renew and to increase the variety offer. Leading multipurpose varieties have continuously declined to leave the place to varieties more specifically adapted to the processing industry and to the modern distribution. Niche markets of yesterday have turned today to major ones (washing, cold storage, firm flesh) or to emerging ones (organic).Intensification and diversification of cultivation areas are strongly pushing the promotion of new varieties that may adapt to the variability of pest and disease pressures and soil and climate changes as well.

In the "potato emerging countries", the 4000 or so known varieties worldwide might not be enough to satisfy all of the diversity of growing and retailing demands. But once again, although some extreme conditions (e.g.: hot climate, salted water) might be more challenging, the breeders will succeed in breeding adapted varieties as they did in the past to adapt potato to the North America and Europe. The true challenge is time to succeed.

Challenge of yesterday, to gather favourable characteristics in a single variety, has been globally honoured by breeding multi-purpose varieties that have local or national adaptations. Today, to gather more and more favourable characteristics, including wide adaptation, without any failure, in specific varieties, is an even more stressful challenge! Breeders must carefully rank and find the best balance between antagonist priorities that come from the growing sector, looking for more and more diversified agronomic adaptations, and these that come from the processing industry and the modern food distribution, looking for higher and higher quality standards of a rather limited number of traits.

Challenge on "How?"

For decades, in public research institutions, the potato has been the object of numerous academic researches in almost all plant sciences. Potato breeders have been fortunate to be able to rely on for their applied research.

Potato has acquired a status of a model crop in the development of the modern biotechnologies that have opened roads to the geneticists for fascinating, diversified applications, concerning the resistances to pests (insects, nematodes) and diseases (fungi, bacteria, viruses), the processing qualities (content and quality of the starch, reducing sugar) and cooking qualities (alkaloid content), as well as medicinal compounds (serum albumin). Although only a few of them have had economic impacts (e.g. micro-propagation and the others still face huge problems in tentative transfer of the technology to the seed and breeding industries (protoplast fusion, GMO), potato breeders must acknowledge the strategic importance of the fundamental research.

But the complexity of the genetic structure and of the plant physiology, the duration and the difficulties of the evaluation of the phenotype, have resulted in slow progress and a declining scientific attractiveness of the potato. Arabidopsis, Rice and Tomato are today much more valuable to the young scientists. Public research institutions are moving out of the potato research (e.g. PBI, PRI, MPI, INRA). In EU, over production has been the rule for so many years that potato is no longer considered as the strategic crop that it used to be. All member states are in the process to reduce funds and grants to research and production as well.

As a result, easy access to scientific information and expertise that potato breeders have been used to get from the public institutions has been declining very fast! Few private companies have been more and more engaged in fundamental potato research, either alone or in cooperation. Unfortunately, most of the potato breeders are just not “big” enough to do so. Even the “traditional” leaders cannot compete with the large biotech companies.

From the early 80s, personal computer technology has been a major innovative tool to improve the huge data management task of potato breeders! Moreover, the Web provides a nice facility to share the scientific, technical or economical information that is no longer available at the national level. The development and the maintenance of the CGN and the EUCABLIGHT networks are examples of powerful initiatives. Still, private breeders should not leave the scientists alone and they have to bring their support in the management of the gene banks.

Challenge on Laws!

Since 1961 UPOV international Convention has given the breeders access to intellectual property rights to protect the commercial exploitation of the new bred varieties. Although the system proved to be highly efficient in the “Old World” and although most of the countries are today members of UPOV, the implementation of their rights is quiet a new big challenge.

Until recently, potato breeders looked at the counterfeited production as being marginal and without any big commercial impact. But today, it comes out that the added value of a few new protected varieties compared to the old public ones is so attractive that indelicate growers and merchants developed the illegal production to high level!

National laws, although in agreement with UPOV Convention, provide the breeders with too many different legal instruments that is almost impossible to implement their rights in many countries. Transcription of the legal provision for farm saved seed of the UPOV 1991 Convention into the EU Reglement & Directives is another example to illustrate how bad the situation is.

The Rio Convention on Biodiversity and the FAO Treaty on Plant Genetic Resources might be new challenges to come to the breeders by introducing restrictive international rules for the germplasm exchange that has been quiet fair and simple so far.

Conclusion

Plant Breeding is anything but an innovative and integrative activity that is increasingly complex. Potato breeding is facing big changes in its scientific, economic and legal environments. These may result in challenges that breeders have long been trained to deal with, say new traits to bred for or new technology to use, but also in challenges that require the development of new qualifications as potato breeding have to be more involved in basic science, to be more receptive to public concerns and to be more proactive in legal things to defend its rights.

POTATO CROP NITROGEN STATUS (CNS) ASSESSMENT TO IMPROVE N FERTILISATION MANAGEMENT AND EFFICIENCY

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Introduction

The effects of excessive or insufficient nitrogen application of N fertilizer to potato crop are well known on tuber yield and quality, but also on the N losses that can occur to the environment.
So there is a clear need to convince potato farmers to apply the right N fertiliser rate in the right time and at the right place to maximise income, and to stay within the constraints of expected tuber quality and respect of the EU nitrate directive (91/676/EEC). Recent increase in N fertilizers prices should also significantly drive farmers to more careful input management.

With regard to apply the whole recommended amount of N fertilizer at planting, it is generally agreed that the establishment of a field-specific N recommendation for potato at planting time can never be accurate as it is not possible to predict with precision the total crop N requirements and soil mineral N supply that will occur during the growing season. Such variables are influenced by several predictable or unpredictable factors, such as weather conditions, chemical and physical soil properties, type and evolution of organic matter previously incorporated in the soil, cultural practices, variety earliness and crop duration.

N fertilization strategies that combine splitting of N fertilizer recommendation with the assessment of crop N requirement during the growing season can largely help to better match needs and supply, and as a consequence to improve N fertilizer efficiency (Vos and Mackerron, 2000).
The considerable advances that have been recorded the last decade in the understanding and modelling of N cycle transformations in the soil-crop system and also in the modelling of potato N-uptake and potato growth can be integrated in such strategies. At the same time, continuous improvements arise in the technologies useful for in-season monitoring of CNS. Such tools are useful to fine-tune crop N requirement and consequently can help to decide on the need for supplementary fertilizer N application as top-dressing.

Combination of on the one hand modelling approach to establish a global N recommendation at field-specific scale and on the other hand CNS monitoring tools can lead to the building of Decision Support System useful to optimise N fertiliser rate.

The use of CNS assessment is based on the consideration that instead of attempting to finely predict mineral N supply from the soil, it may be simpler to rely on the crop itself to indicate its N status in order to respond accordingly. Crops are indeed often considered good integrators of growing season conditions factors such as the presence of mineral N in soil, the weather and crop management (Schroder et al, 2000).

This abstract paper deals with the state of the art and potential development on characteristics, use and implementation of well-known and more recent methods aimed to assess in-season potato CNS. The main objective remains to improve the management of N-fertiliser and its efficiency through the integration of CNS assessment into DSS. Such an approach asks to deal N fertilisation management towards areas with sub-optimal growing conditions for N availability, particularly by avoiding excess N application at planting time (MacKerron, 2000).

Methods to assess potato CNS

What is CNS? The N status of a crop can be described as the comparison of the current nitrogen content in the crop to the critical crop N content required to avoid crop N deficiency inducing risk of yield losses. CNS can be assessed either through plant N concentration (expressed in percent of N in the dry biomass) or crop N uptake (expressed in kg N per ha of soil area) determination.

On this base, methods aiming to monitor CNS have to lead directly or indirectly to a good evaluation of whole crop N content. Ideal basic requirements for such methods are:

- *Sensitivity to N*, which means that early detection of deficiency in CNS should be possible
- *Specificity to N*, which means that the measurements should ideally be exclusively related to nitrogen supply and to plant N concentration, without interference from other external factors
- *Accuracy / precision,* so that the measurements related to N status are reliable and repeatable.
- *Feasibility*, meaning that if wider use of the tool is wished for farmers or consultants, it should be easy to employ, cheap and fast to provide results.

Potato N monitoring can be operated at different scales: plant tissues, leaf, plant and canopy. In *plant tissues* most used methods are Kjeldahl digestion, Dumas Combustion, NIR spectroscopy and Ion specific potentiometry. These methods reviewed by Haase et al (2004) are generally expensive, labour intensive and not suitable for on-field monitoring. A method based on the measurement of leaf petiole sap nitrate concentration, however largely investigated and applied, has a high sensitivity but low specificity method, and with low precision across the growing season (MacKerron et al, 1995).

At *leaf scale*, among several non-invasive methods reviewed by Booij et al (2000), measurements with a chlorophyll meter device can relate to leaf chlorophyll concentration. Such readings show low specificity and sensitivity except for non N fertilized plots compare to fertilized ones, so that it can only detect deficiency situations for N (Gianquinto et al., 2004). It shows however accuracy and precision for a part of the growing season, particularly with standardized readings on specific leaflet of a main stem taken between a specific time and growth stage interval (Goffart et al., 2002)

At *canopy scale*, most of the usable crop monitoring methods are non invasive as they rely on measurements of light transmitted below the canopy or reflected above. They belong to remote sensing methodology (based on spectral canopy characteristics) that can be operated at different spatial scales: ground-based, airborne or space-borne (Tremblay 2004, Jongschaap, 2006). They are all targeted at the estimation of canopy structure parameters mainly LAI (leaf area index), based on the knowledge that plant N, leaf chlorophyll and LAI are strongly related variables. Above canopy crop N monitoring methods appear to have the largest potential in the near future due to their speed, feasibility and lower cost per unit sampled area (possibility of sampling of larger areas in shorter time than any other technique). However airborne or space-borne methods need to be developed for a large number of users to be economically applicable, and the raw information they supply on canopy N status through the calculation of vegetation indices still requires specific and long image pre-processing time to be useful for crop N management strategies. Moreover sensitivity, specificity, accuracy and precision of these methods are still under investigation. Most commonly tools under investigations for ground-based remote sensing in recent years are the "Cropscan" field radiometer system (Booij and Uenk, 2004), the "N-sensor" a canopy-reflection-based system with four tractor-mounted sensors (Link et al, 2002) or the "Greenseeker" in the US (Tremblay, 2004). Canopy fluorescence is another technique under investigation for crop N monitoring (Tremblay, 2004). Inversion of canopy reflectance models should open larger possibilities for CNS (Casa & Jones, 2004).

It must also be noted that for methods at leaf or plant scales, the link to CNS is not always obvious because sample sizes (a few plants, 20 to 30 petioles or leaves) are very restricted comparatively to the mostly large field areas (several hectares) that are to be investigated by this way. So the scaling up of the assessment of the CNS from leaf to canopy is expected to give a better "average" monitoring result over the whole field. A big advantage of reflectance measurements (either ground-based or air/space borne) is that they represent an integrated measure of nitrogen contents over total canopy depth, which gives direct values for CNS (Jongschaap, 2006). Comparatively, leaf chlorophyll meters measurements only gives information on the top leaves of the canopy.

Regarding spatial CNS variation across a field, another important improvement in the monitoring of N should come by dividing the field into supposed "homogeneous blocks", that will allow to further manage in individual ways parts of the field for supplementary N application.

Use and implementation of crop N monitoring methods in DSS for potato N fertilisation management

Relative vs. raw CNS values

As already mentioned, measured values generated by crop N monitoring tools are far to be specific to N because of the interaction of other factors such as soil characteristics, stages of growth, leaf position, climatic factors, drought, irrigation, diseases,… So an important statement that is now mostly recognised by scientists for use in crop N monitoring methods, is that in order to alleviate the influence of factors unrelated to crop N requirements in the use of chlorophyll or vegetation index (from canopy reflectance) estimation, the use of reference plot is required (Schroder et al, 2000; Meier et al, 2001; Raun et al, 2002) . It is an internal reference plot of the investigated field, either a "zero N" or a "well or over-fertilized" area located in a representative part of the field. Through comparison of the whole field with the reference plot, relative values are used rather than raw values that are identified to be useless for CNS monitoring.

Threshold values

To be useful in strategies aimed at deciding on the need for in-season complementary N, relative values of CNS need to be compared to threshold values, i.e. critical value or range, varying during growth and below which crop production is limited, due to insufficient crop nitrogen content. For most of the methods, either invasive or non-invasive ones, raw threshold values have been determined on yield base (Gianquinto & Bona, 2000). So these critical values are also site and variety specific and are influenced by the same factors as those already mentioned. Only few have been determined and validated for the potato crop taking into account relative threshold values (Olivier et al., 2006).

Combination of the CNS values into DSS

Such relatives values can be used by themselves in combination with N balance models, as described by Goffart et al. (2004) in a DSS using Azobil software and HNT chlorophyll meter readings. Azobil software is a provisional N balance based model at the start of the growing season, to estimate total crop N requirement according to a foreseen yield. Another attempt proposed by Booij and Uenk (2004) uses ground-based reflectance readings with Cropscan to supply current CNS compared to set points for CNS during crop growth.. The major limitation of this interesting approach is the use of raw readings of reflectance, instead of relatives ones. Supplementary N amount is however calculated.

CNS values can also be integrated into DSS based on crop growth simulation models that enable timely and quantitative prediction of the dynamics of crop requirements for a specific location. Possibility and interest of integration of such models with remote sensing readings at potato canopy scale for CNS assessment have been deeply investigated by Jongschaap et al. (2006).

A final and important approach is the use of CNS values in the frame of precision agriculture. Generally, the use of such values to guide decision on the need for supplementary N considers the field as an homogeneous entity which of course will never or rarely be the case. So the concept of variable nitrogen rate application (VNR), which can be required to optimize N management in fields with variable soil properties (i.e. soil mineral N supply), should be combined with the used of CNS assessment. Zebarth et al. (2003) have investigated the possibility to use the N-sensor for a real-time mapping of spatial variation in potato N status, so that to fine-tune in season fertilizer N application based on potato N requirement. Note that the use of over-fertilized windows to generate relative value of crop reflectance are suggested by Link et al. (2002) for the use of N-sensor system, while it has been shown to be less effective than a non-fertilized N window.

Perspectives

For the potato crop, reliable DSS combining N-balance models or crop growth simulation models with tools for in-season CNS monitoring are under investigation. These will tend potentially in a near future to improve N fertilization management and efficiency through a better positioning of N supply in space and time according to crop N requirements.

The widely adoption of such systems will require tests and validations under a wide range of climates and agronomic situations. It will also require the general use of relative reading values, for observed as well as for threshold or critical values or ranges for N deficiency. However, specific relative thresholds values should nevertheless be established for different varieties or groups of varieties and checked for irrigated vs. non-irrigated conditions (Goffart et al, 2004). From the different methods developed since years for CNS assessment, above crop reflectance measurements are potentially the more promising, mainly because they can integrate canopy depth and larger canopy areas than any other methods. However their feasibility, accuracy and sensitivity still need to be investigated more deep inside. A challenge in the use of such methods still remains the accurate assessment of the size of the supplement N that should be given to the crop when a deficiency is found to be present or imminent.

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ADVANCES IN MODELLING THE POTATO CROP: - SUFFICIENCY AND ACCURACY CONSIDERING USERS, DATA, AND ERRORS

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Mathematical models are required to describe complex, multi-step processes such as crop growth or progress of a disease. The commonest form in use is the simulation model although there are other forms such as Rule-based, or Bayesian, or 'fuzzy', depending upon the application. Models are now being combined into packages that we call decision support systems. Mathematical models of the potato crop have been devised, in a range of sophistication, for some time and lead to the quite proper questions, "Are they suited to their applications?" and, "Where next? What are the developments that are sought or that are needed?"

Simulation modelling can have any one of several purposes. One, not often claimed, is to formalize our knowledge. In my opinion, many published mathematical models fall into that category. Why? Because most uses of models carry constraints that make many, otherwise excellent, models quite inappropriate. Some models require too many parameters for them to be used in anything other than further scientific study. A valid scale for inputs and outputs can be another constraint. Uses may include provision of a yardstick against which to compare real crops and the simulation of real, constrained crop growth to monitor and avoid the onset of stresses. Sensitivity analysis of the crop as described by a model can be used to estimate the potential for crop improvement by breeding (e.g. Jefferies, 1993; Haverkort & Grashoff, 2004) or by cultural manipulation (e.g. Jamieson et al., 2004).
Each of these possible applications carries its own set of features and constraints such as the time-scale that is meaningful, or the spatial scale that is appropriate. For example, yield is a common output, with values being cited at the scales of plant, plot, field, parish, political region, and nation. At each of those levels the input data that is available and the knowledge of the system that allows parameters to be quantified differ so that no one model can be appropriate for all applications.
Where a model is reasonably successful in its aims, it may also be used in forms of decision support whether strategic: – How much water should I licence? – Or tactical: – Should I irrigate today or tomorrow? A simulation model of the development of a pathogen and its interaction with a crop provides similar applications (e.g. Raatjes et al., 2004). - Is it time to re-apply blight spray? There are many such possible uses and in all of them there are constraints and features of scale.

Other uses of models include their combination with other models to examine properties of a system such as crop rotation, or they might be used to estimate consequences beyond our experience e.g. climate change (Wolf, 2003).

The appearance of a model

Growers and agronomists will only run models of crops and pathogens if the rewards are worth the input of time and energy. The modeller has to consider how the model will appear to the user and how the user will provide the inputs required. To answer these and related questions the development of any model should include re-iterative consultations with the intended users. A related question is whether a model should be 'free-standing' or whether it should be wrapped in a package that would allow it to be used in an internet-based system.

Characteristics of crops and models that must be reconciled

Crop data and cultivar characteristics:- Cultivars differ in more ways than those that are characterised in national lists of cultivars. Examples include the base temperature for sprout growth, maximum leaf size and the extent of root growth. These present a very practical problem for both the modeller and the user. If the characters are needed for simulation of the crop, should they be included even though the user may not be able to provide values for the parameters, or should the model be given 'typical' values to characterise it for all cultivars or for a group of cultivars? Then should / can the user accept the inaccuracies.

Another example to be discussed is the ramifications of the physiological age of the seed.

This example characterises the differences between the modeller and experimentalist on the one hand and the agronomist and farmer / user on the other.

Soil data and soil conditions: - Soil water capacity and the characteristics of moisture release are dependent upon soil physical characteristics that differ vertically in the soil profile at any one place and that differ horizontally. These present problems of spatial scale which will be discussed.

The nitrogen that is available for uptake by the crop can modify rates of leaf expansion, the onset of senescence and maturity. Fluctuation of available soil N through the growing season poses a problem of temporal scale which needs to be considered.

Growth of the crop: - The uptake of water by plant roots is dependent upon the growth of the roots and also on the relative locations of the roots and the water in the soil. Some models aim to by-pass this problem. Others have represented root growth in very different ways with consequences for the reliability (and the comprehensibility) of effects.

The, simulation of dry matter partitioning to tubers is an instance where models may be planned to give the 'right' answer (under certain conditions) without truly reflecting the mechanism (Kabat et al. 1995).

Weather data: - Provision of weather data is a key feature in the operation of models of crop growth and of disease development. Modern automatic weather stations are a realistic means for growers or groups of growers to obtain the weather data that they will require to run such models but they do represent a significant cost in both time and money. By and large, the difficulty and cost of obtaining adequate weather data is the Achilles' heel of the introduction of useful models to growers and agronomists at the present time.

Problems of scale:

Time - The earlier models of crop growth (e.g. De Wit, 1965; Ng & Loomis, 1979) and some more recent ones (e.g. Kadaja & Tooming, 2004) simulated the basic processes of crop growth, photosynthesis and respiration, which operate on time-scales of seconds, and built up on these to quantify dry matter production from records of temperature and incident radiation. These give problems of time-scales and the use of averaged data and huge number of iterations. Many later models suffer equivalent problems.

Space - Many of the relations that are described by functions within simulation models have been determined on single plants or even on individual organs e.g. rates of sprout growth and leaf growth. Yet the purpose of an application of a model may be to describe the growth of a whole crop of one to twenty hectares, or even more. The purpose could even be the estimation of a national yield.

The issue of scale is not that there is a right and a wrong way to model. Rather it is to devise and use a model with scales appropriate to the use that is intended.

Foreseeable developments in modelling

The combination of models into coherent packages has been used in both Europe and North America and is likely to be a significant development. One example is MAPP, the Management Advisory Package for Potatoes, from the UK, and introduced in 2001 (MacKerron, 2004) which combined a simulation model of crop growth with a rule-based model of tuber size distribution to offer a means to manage a potato crop through a single season, offering recommendations on seed rates and harvest dates in the light of the cost of seed and the price for the harvested crop. That package may be upgraded to extend the range of its environmental validity and its cultivar data, as well as to incorporate further models within the package. Another package, OPTIRas, is currently under development in collaboration between Scotland and the Netherlands to combine models of cultivar resistance and tolerance to PCN with a model on tuber size distribution and, possibly, with other models simulating other parts of the cropping system.

A third example is GUICS, a 'graphical user interface for crop simulators' developed by the ARS of the USDA to facilitate the interfacing of differing models dealing with crops and / or the environment (USDA, 2007). A specific example of the use of GUICS was the development of 2DSPUD by the combination of models of soil and of potato growth. The model has been released to farmers for input optimization and farm management. It should be worth watching for reports of the further development of this system.

Conclusions

Modellers should be encouraged to join in an exercise to define the domains of validity of their models.

The combination of models into packages that address a range of problems is a more useful approach than creating single, large models that will do everything.

The purpose of this paper will be to examine the constraints on the models, imposed by the applications; and the limitations upon the applications that are imposed by the requirements of the models.

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PHYSIOLOGY OF THE POTATO: NEW INSIGHTS INTO ROOT SYSTEM AND REPERCUSSIONS FOR CROP MANAGEMENT

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INTRODUCTION

Observation of root system under field conditions is tedious work consuming a lot of time and labor. Many potato men, however, have worked actively and patiently to clarify the length and dry weight (DW) of roots, their distribution throughout soil profile, and the relation with shoot and tuber growth. In this presentation I will explain the present attainment of the knowledge for potato root system, and discuss the implication on breeding work and crop management.

ROOT LENGTH DISTRIBUTION

The distribution of root length throughout soil profile under field conditions was examined in several countries, e.g. Vos and Groenwold (1986) in the Netherlands, Stalham and Allen (2001) in UK and Iwama et al. (1993) in Japan. These researches revealed the general feature of potato root characteristics in details. The roots are concentrated mostly in the plow layer above 30 cm in soil depth, even under mother tubers, but some roots extend until below 100 cm depth and total root length throughout soil profile reaches about 10-20 km per one square meter of planting area. In a comparison between potato and other field crops, Yamaguchi and Tanaka (1989) confirmed that root distribution in potato is much shallower and less dense. In their measurement the total root length throughout soil profile was 21 km/m^2 in potato, while 86, 78, 52, 50 and 39 km/m^2 in wheat, rice, sugar beet, maize and soybean, respectively.

GENETIC DIFFERENCE

A large genetic difference was also found in potato root mass. Root DW in plow layer at the maximum shoot growth stage (almost the end of flowering) varied 1.1 to 5.4 g/m^2 in 10 varieties (Iwama et al. 1979), 0.3 to 19.4 g/m^2 in 268 clones (Iwama et al. 1981a), and 3.4 to 40.9 g/m^2 in 9 potato species (Iwama and Nishibe 1989). In root length throughout soil profile at the maximum shoot growth stage, the difference was 4.8 to 7.3 km/m^2 among 4 varieties (Iwama 1998), and 7.8 to 24.1 km/m^2 among two varieties with three planting densities (Iwama et al. 1993). In addition, in the comparison between 6 populations derived from the crosses with two female parents and three male parents (Iwama et al. 1981b), root DW in plow layer was significantly larger in the population derived from the parents with larger root DW. These results suggested that genetic improvement of root mass is possible and the resources for breeding are plentiful within potato species.

RELATIONS OF ROOT MASS WITH SHOOT AND TUBER GROWTH

Under favorable environmental conditions without severe shortage of water and nutrients, the difference in root mass between genotypes is relating with the maturity class, i.e. late genotypes continue the root growth longer, and attain larger root mass and deeper root distribution than early genotypes (Iwama 1998, Stalham and Allen 2001). Therefore, maximum root mass during the growing season generally shows positive correlations with maximum shoot mass and final tuber yield in the comparisons between varieties (Iwama et al.1979, Iwama and Yamaguchi 1996) and breeding clones (Iwama et al. 1981a) of different maturity classes. In addition, these differences in root mass become clear at the start of flowering, much earlier than those in shoot mass, and are relatively stable over locations (Iwama et al. 1980). The physiological relationships of root mass with shoot and tuber growth were also revealed experimentally. In root pruning experiment (Iwama and Yamaguchi 1996), the reduction of root mass resulted in the decrease of leaf growth and nitrogen absorption, and final tuber yield. In grafting plants between two varieties with different root mass (Iwama et al. 1995), early tuber bulking was negatively related with the increase of root mass.

THE GENETIC IMPROVEMENT OF ROOT MASS

The most frequently reported reason for high sensitivity of potato to water stress is its shallow root system (Harris 1978, Loon 1981) and low root length density (Gregory and Simmonds 1992). Two breeding attempts to increase root mass and/or root depth were done in Peru (Ekanayake and Midmore 1992) and Japan (Iwama et al. 1999). Ekanayake and Midmore (1992) used pulling resistance of roots (PR) as a selection criterion of root penetration ability into deep-soil layer. The PR showed a significant positive correlation with maximum length and DW of both pulled roots and residual roots in plow layer at drought sites. Iwama et al. (1999) selected 4 genotypes using selection criteria of root DW in plow layer and final tuber starch yield from the cross between Irish Cobbler (early cultivar) and Konafubuki (late cultivar having high starch content but small root DW) under rain-fed conditions without severe drought. In the later experiment in the field with severe drought under rain shelter, the selected genotypes revealed greater root DW not only in plow layer but also in deep-soil layer than Konafubuki, and showed significantly less reduction of leaf conductance and photosynthesis, leaf area index, and final tuber dry yield.

FUTURE PERSPECTIVE OF PHYSIOLOGICAL RESEARCH FOR ROOT SYSTEM IN RELATION TO BREEDING AND CROP MANAGEMENT

Water absorption of roots from soils is affected by many aspects, e.g. soil characteristics relating to both content and potential of water, climatic conditions such as temperature, solar radiation, humidity and wind, and plant characteristics such as leaf area and growth stage. Therefore, quantitative relationships of root mass and/or extension depth with water absorption are very complicated and have not yet been clarified in details. In the present decade, however, new devices easily and cheaply to measure soil water content and soil water potential in fields have been developed. By coupling with root length measurement, we may be able to realize the relationship between root length distribution and water absorption in fields. New methods are also being developed to evaluate genetic difference in root characteristics, such as root extension ability to hard soil layer using pots with paraffin-Vaseline discs (Gopal et al. 2006), and water absorption ability in roots under low water potential in vitro (Gopal and Iwama 2007). These new methods may make possible to improve genetic potential in water absorption ability of roots from deep-soil layer and dry-soil layer. In addition, if we use a DNA mapped population in these measurements, QTL analysis may reveal the relating DNA makers to these characteristics and make possible indirect selection of roots with DNA makers. In an aspect of crop management, an accurate time and frequency for irrigation will be clarified by the measurement of both content and potential of soil water at the same time, and contribute to economize irrigation water. Breeding of new varieties with excellent root system to absorb water from deeper soil depth and under lower soil water potential will also increase the usage of soil water stored or irrigated and contribute to more efficient utilization of water for potato production.

In conclusion, physiological researches for potato root system performed in the past and being performed in the future, contributed and will contribute for the development of new cultivars with high drought tolerance and the improvement of irrigation practice. Close cooperation between researchers in physiology, breeding and crop management is desired for potato production in the world.

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Oral Presentations

Genomics

EPITOPES OF THE POTATO VIRUS Y (PVY) COAT PROTEIN RECOGNIZED BY MONOCLONAL ANTIBODIES

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Potato virus Y (PVY) strain groups are based on host-response and resistance-gene interactions. The strain groups PVY^O, PVY^C and PVY^N are well-established for the isolates infecting potato in the field (reviewed in Singh et al. 2008). The genes for hypersensitive resistance to PVY^O and PVY^C are rather common in potato cultivars and protect, with varying efficiency, potato plants against systemic infection with PVY strains belonging to the corresponding strain groups. However, these cultivars are susceptible to PVY^N. Therefore, there are practical reasons to distinguish PVY^N strains from those of the two other common PVY strain groups. There are commercially available monoclonal antibodies (MAb) that recognize differences in the coat protein (CP) of PVY. The epitopes for them, however, are often unknown, which hampers possibilities to make predictions on how globally they recognize or distinguish strains and isolates of PVY. Such predictions will be increasingly feasible with use of sequence data accumulating from the CP-encoding sequence of PVY isolates. The aim of this study was to map the epitopes of three commercially available MAbs provided by Adgen (Neogen).

The CP-encoding sequences of PVY isolates PVY^N-605, PVY^C-Adgen and PVY^O-Sasa were used to design a series of 20 amino acids long peptides, each with a 3 amino acid residues long sift as compared to the previous one. Hence, there were 84 peptides to cover the CP of one strain. These peptides for all three strains were synthesized each as a spot on the same SynthoPlan APEG CE Membrane, as described (Frank 2002), using a fully automated MultiPep Spot robot (Intavis AG). The membranes were probed with Mab1128, Mab1129 or Mab1130. Binding of the MAb was detected using horse radish peroxidase conjugated anti-mouse rabbit antibodies and visualized using enhanced chemiluminescence (ECL) and autoradiography on X-ray films, as described (Kaikkonen et al. 1999). The epitopes were further defined in the MAb-binding peptides by alanine-scanning and amino- and carboxyterminal deletions.

Epitopes for all three MAbs mapped within the first 30 N-terminal amino acid residues of the CP. Mab1128 detected five peptides of strain PVY^N605. Mab1129 detected five peptides each of the strains PVY^O-Sasa and PVY^C-Adgen but no peptide of PVY^N, and the detected peptides corresponded to the same positions of the CP in the two strains. Mab1130 detected two peptides from the same positions of the CP in all three strains. Further experiments to define the epitope sequences in the detected peptides indicated that Mab1130 bound to an epitope IDAGGS conserved in all three strains, which is consistent with detection of viruses of all the three PVY strain groups with this MAb. Mab1129 bound to an epitope RPEQGSIQSNP found in PVY^O and PVY^C. Mab1128 was specific to PVY^N and detected an epitope NLNKEK. Alignment of the available PVY CP-encoding sequences in public databanks showed that the aforementioned amino acid sequences are either common to all PVY strains (Mab1130), found in PVY^O and PVY^C but not PVY^N (Mab1129) or characteristic of only PVY^N (Mab1128). These data suggest that the three MAbs are useful for distinguishing PVY^N from PVY^O and PVY^C, or for detection of all strain groups, at the global level. While the differences of the CPs are not fully correlated with the biological differences of PVY strains (Singh et al. 2008), detection with these MAbs will serve as a useful preliminary characterization of unknown isolates of PVY from the field.

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TRANSCRIPT PROFILING OF THE EARLY EVENTS ASSOCIATED WITH POTATO TUBER COLD SWEETENING: AN HETEROLOGOUS MICROARRAY APPROACH

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The early events triggering potato tuber cold-induced sweetening (CIS) are largely unclear. Since no high-quality, standardized microarray platform is presently available for potato, we conducted an heterologous hybridization approach with the commercially-available tomato Affymetrix GeneChip interrogated with potato tuber transcripts (4 days at 4°C versus 17 °C control). Cold-responsive genes were confirmed and further profiled in detail by Real-Time-qPCR over a 26-d time-course. Our combined GeneChip-qPCR data identify at the sequence level enzymatic activities long known to take part to CIS, as beta-amylases, and disclose further unexpected transcriptional processes as induction of redox- and ethylene-associated genes.

Introduction

Affymetrix has not yet developed a potato GeneChip, although spotted cDNA arrays (e.g. TIGR) have been available for potato for some time. However, TIGR Potato cDNA Microarray distribution has recently been discontinued. Potato and tomato appear to be suitable for conducting a heterologous approach as the two Solanaceous species are strictly phylogenetically related [1]. Here we describe the development of an approach [2] based on the generation of a list (Global Match File) where tomato GeneChip target sequences are aligned to potato EST-derived clusters/singletons (TIGR TA).

Our heterologous approach was conceived as a pre-requisite step for investigating potato tuber cold-induced sweetening, which has been studied for more than 120 years since its first description [3]. Incubating potato tubers at 2-8°C causes the accumulation of sugars (mainly sucrose, glucose and fructose) at the expense of starch, and is therefore detrimental for tuber quality despite delayed tuber sprouting [4]. Furthermore, upon cooking, dark, bitter tasting melanoidins are produced by Maillard reaction involving reducing sugars (as glucose and fructose) and free amino-acids [5]. In recent years, there has been increased concern since a specific type of Maillard reaction involving the amino-acid asparagine (abundant in potato tubers) and reducing sugars has been shown to produce the genotoxic and neurotoxic compound acrylamide [5]. Since the discovery of cold sweetening, the various genes playing a key-role in the carbohydrate metabolism from starch degradation to sucrose synthesis and breakdown have been investigated [4]. However, the precise contribution of each gene is still unknown and there is no detailed overall picture of the early events triggering cold sweetening. This could be in part attributable to the heterogeneity of the systems used (tuber varieties, experimental settings, etc.) and is an unavoidable drawback of low-throughput expression profiling techniques that focus on one or a few genes that code for well-known enzymes.

Some agreement exists on the following early, cold-induced events as being causative/associated with cold sweetening. At least one amylase activity, demonstrated to be beta-amylase based on substrate specificity, was visible very early in iodine-stained zymograms [6, 7]. Sucrose phosphate synthase (SPS) underwent a change in kinetic properties and its transcript was induced in a few days at temperatures of 5- 3 °C [7]. UDP-glucosepyrophosphorylase (UGPase) transcript level was high in developing tubers but an increase was also evident upon cold incubation of tubers [8]. Also, specific UGPase isoforms may be associated with increased susceptibility to cold sweetening [8]. Acid invertase was observed as playing a critical role in reducing sugar accumulation and its transcripts were found to be strongly induced after a few days at 4°C; however, the total amount of invertase activity was found to be solely related to the hexose/sucrose ratio rather than total reducing sugars [9].

In this study, apart from investigating heterologous GeneChip approaches, our main aims were: (i) to identify early cold-responsive gene family members to be studied in more detail by qPCR over a 26-d time course; (ii) to obtain an overall transcriptional picture of these genes, including those previously studied, taking advantage of the use of a single, standardized system (i.e. same genotype and experimental settings); (iii) to obtain a crude, global view of early expression trends (as related to sugar accumulation) for those genes that are not carbohydrate-associated.

Results and discussion

The Global Match File

A Global Match File was generated in order to address the following issues: (i) considering a given tomato probeset, which (if any) potato transcript(s) are reliably represented and what is the quality of this association; (ii) retrieval, for such a potato transcript, of an updated and high quality annotation (based on TIGR TA); and (iii) generation of subsets of "highly reliable" probesets whose alignment scores to potato counterparts are above a tuneable threshold (e.g. 90% or 70% alignment thresholds) and thus can be considered with confidence. The Global Match File consists of 269,474 alignments and has several indexes for assessing alignment fidelity among tomato and potato sequences. In particular, some further parameters were developed in order to evaluate if alignment interruption is due to the tomato target region being designed over a stop codon (data not shown).

Sugar accumulation

The pattern of early sugar accumulation was studied in a time course experiment spanning 26 days. Day 0 represents the 17 °C control. Sucrose, glucose and fructose contents of three independent tubers for each time point were measured and their averaged values ± SD are plotted in Figure 1. Sucrose began accumulating at the onset of cold incubation and reached a plateau within two weeks. Glucose and fructose accumulated slowly during the first few days and increased sharply as the sucrose levels stopped increasing. At the last sampling time (day 26) glucose and fructose levels equalled sucrose concentration on a molar basis.

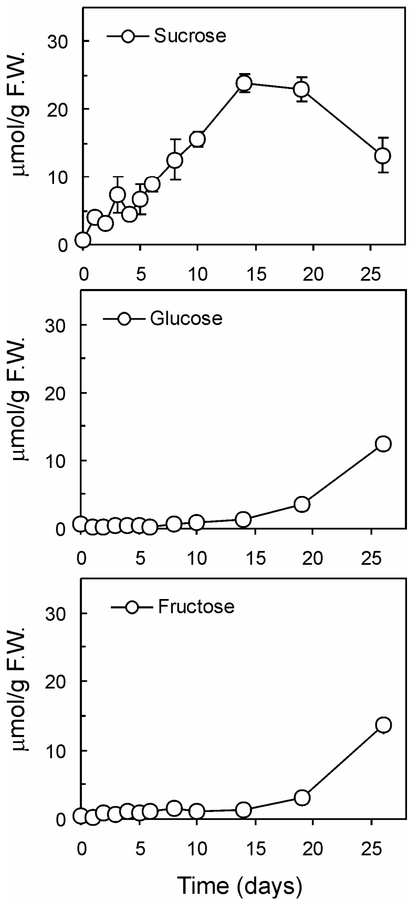


Fig. 1. Sugar accumulation

GeneChip data

Two biological replicates were used by extracting total RNA from two distinct tubers for both the control (17 °C) and cold treatment (4 days at 4 °C). Microarray analysis was performed using R/Bioconductor. Expression measures for each probeset were obtained using GCRMA. The dataset revealed 1,854 differentially expressed genes. Several cold-responsive genes, which were further studied in detail by qPCR over a 26-d time course (see next section), were identified. Among the global expression trends triggered by cold we observed stimulation of carbohydrate-associated genes (CAG), flavonoid, redox and ethylene-related genes. While enhanced expression of CAG was expected, stimulation of flavonoid associated genes may be a consequence of the raised sugar levels [10]. Ethylene production in potato tubers may be a specific response to chilling ("chilling ethylene" is known for Solanaceous species) or a vestigial response due to relatedness to climateric Solanaceous species such as tomato. Induction of redox and thiol signalling genes may be critical for activation of starch degradation by thiol signalling.

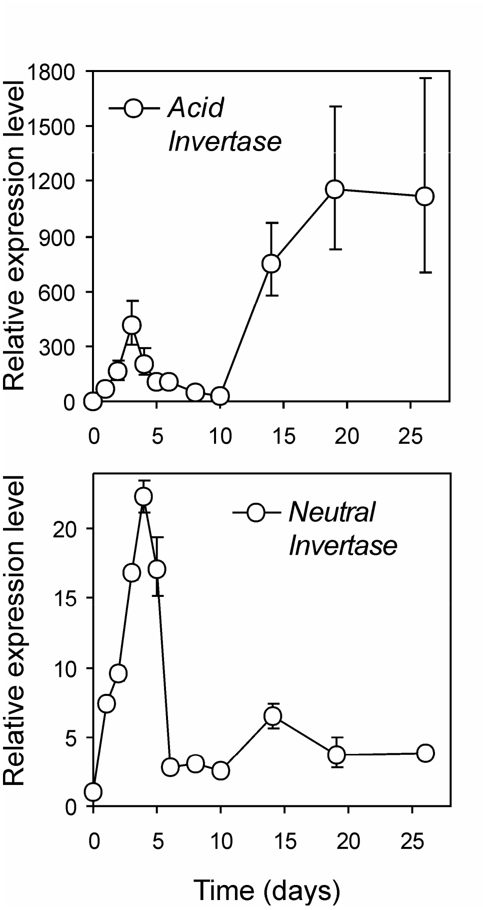
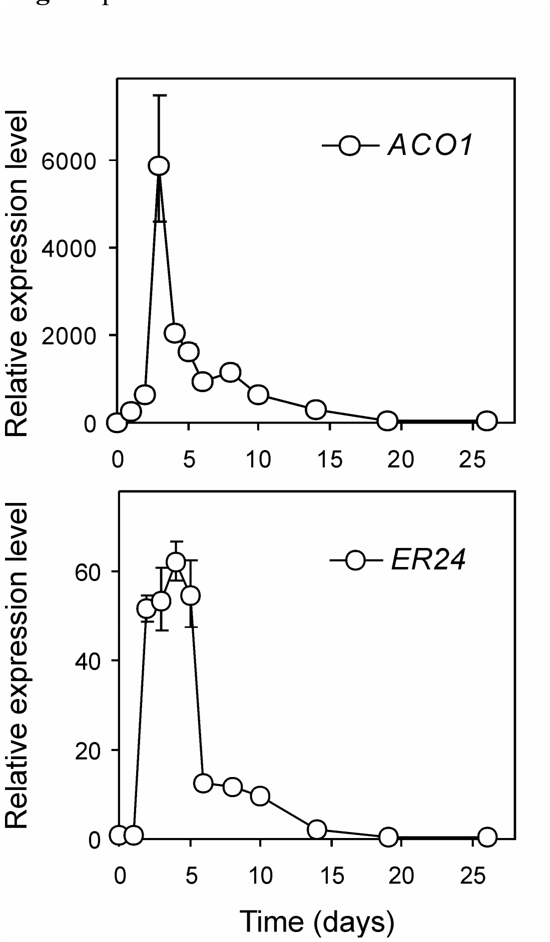


Fig. 2. qPCR of invertases

qPCR Data
Several cold-responsive genes as revealed by GeneChip data were further analysed by qPCR. The amplification was carried out using an ABI Prism 7000 sequence detection system (Applied Biosystems) in accordance with the default ABI Prism 7000 PCR program for PCR conditions. Potato EF1-alpha (AB061263) was used as an endogenous control. Gene-specific primers/TaqMan probes were used. We detected an early, major transcript peak for various cold-responsive genes which had not be resolved before.
This was true also for acid invertase (Fig 2). A further, neutral invertase was shown to be early induced (Figure 2). Figure 3 depicts transcript accumulation of ethylene-associated genes. A dramatic transcript accumulation was detected for ACO1 (ACC oxidase; "ethylene forming enzyme") which peaked at day 3 with a 5,800-fold induction. Transcript levels were subsequently lowered with an intervening shoulder at day 8 which preceded a further decrease. However, at the last sampling time point, ACO1 transcripts were still 43 times higher than the baseline. ER24 (an ethylene-responsive factor bearing similarities to transcriptional co-activators) transcripts increased at day 2 of cold incubation reaching a 60-fold induction until day 6 was strongly upregulated (13-fold).



Based on GeneChip data, further cold-responsive genes were identified and tested by qPCR. In particular, two cold-induced beta-amylases were revealed and identified at the sequence level (data not shown).

Conclusions
The development of tomato-potato Global Match File was helpful in assisting the heterologous tomato-potato hybridization, indicating that similar approaches for other species appear feasible. The use of a ready, highly standardized platform with publicly available probeset data such as the commercially available GeneChip should reduce cross-laboratory differences, which may affect custom array approaches.
GeneChip data for potato cold sweetening suggest that an intricate crosstalk of regulatory molecules including ethylene and sugars is triggered within a few days at the onset of cold incubation. Flavonoid and redox genes show as well enhanced expression. Further research is needed to assess the contributory/causative role of ethylene in CIS.

Fig. 3. qPCR of ethylene-associated genes

The GeneChip-assisted qPCR dataset detects a previously unknown early burst of expression of several carbohydrate-associated genes, including beta-amylases and invertases. Activation of redox machinery suggests that thiol signalling may play a critical role in early cold-sweetening events. At least for the subset of early cold-responsive gene family members represented in the GeneChip, no phosphorolytic starch degradation can be seen from our transcriptional data, while an unexpected accumulation of SuSy transcript is evident in the first days. In accordance with previous investigations, transcript profiling supports a continuative role of SPS and acid invertase in CIS. The identification at a sequence level of various enzymes well-established as playing a role in CIS and the discovery of several unexpected CIS-associated global expression trends provides new molecular and conceptual tools for further understanding this phenomenon. This would then improve our knowledge of key food safety issues such as potato chip darkening and acrylamide content upon frying.

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DETECTION OF CANDIDATE GENES FOR USEFUL TRAITS APPLYING DIFFERENT MOLECULAR TOOLS

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Introduction

Molecular markers are useful to construct linkage maps and to localise monogenic and polygenic traits, allowing the efficient introgression and selection of individuals with specific characteristics already in breeding material. Molecular markers play also a crucial role in the isolation and cloning of plant genes by map-based cloning.

Markers representing concrete genes which influence a trait of interest can be applied directly in marker-assisted selection, independent of the genetic background, and are useful to establish functional maps. Actually, several molecular techniques are available to detect such candidate genes. Theses techniques include cDNA-AFLP, microarray analyses, direct EST sequencing, candidate gene mapping and the construction of physical maps (Ritter et al. 2005).

In previous studies a complete transcriptome map of the potato genome had been established using cDNA-AFLP (Ritter et al. 2008) and cDNA fragments were anchored to the bins of a high-density reference map of potato (UHD map; van Os et al. 2006). Published QTLs can be projected also onto this reference map and cDNA markers (or TDFs) which are co-located (or very closely linked) to these loci could represent potential candidate genes for explaining these QTLs. In our studies we have analysed such co-located cDNAs.

Projection of SSR markers and known QTLs allow also to reduce efforts when analysing the corresponding traits in a new genetic background. All known positions can be screened for the presence of a QTL by analysing only closely linked SSR markers in a new population. We have screened three progenies descending from different resistance sources for selectable QTLs.
We have also applied differential cDNA-AFLP to detect cDNAs which are differentially expressed under biotic and abiotic stress situations and analysed their biological meaning.

The micro array technique allows to analyse the expression of many cDNAs at a time by hybridising cDNAs spotted on microchips with appropriate substrates (McKenzie et al. 1998). We have used microarray analysis to detect resistance or response genes to nematode and P*hytophthora* infections.

Candidate cDNAs which are identified through differential cDNA-AFLP, microarray analysis, direct EST sequencing but also known candidate genes can be integrated in a reference map by designing appropriate primers for obtaining segregating amplification products. In this way the information from different experiments can be integrated in one functional reference map.

Materials and Methods

For mapping candidate genes the UHD mapping population described by van Os et al. (2006) was used. Differential cDNA-AFLP and microarray analyses were performed in a set of accessions of different Solanum wild species described by Ruiz de Galarreta et al.(1998).

For QTL screening the progenies D: CAN 310956.8 x GON703354, E: BUK 210042.5 x PHU81ccc and G: JAM 27521.48 x GON703354 were used.

Nematode inoculations (G. pallida Pa2/3) and *P. infestans* infections were performed as described by Ruiz de Galarreta et al. (1998).

SSR analyses were performed according to Milbourne et al. (1998)

The cDNA-AFLP technique was applied as described by Breyne et al. (2003)

For microarray analyses we used the 10K TIGR chip and the protocols provided by the TIGR institute. Isolation, cloning and sequencing of amplification products were performed using standard methodology (Sambrook et al. 1989).

Sequence homology searches were performed in public sequence databases *via* NCBI using BLAST search algorithms.

QTLs and markers were projected based on common marker intervals in different linkage maps and linkage mapping was performed as described by Ritter & Salamini (1996).

Results

Over 200 published QTLs for resistances and quality traits were projected onto the UHD reference map. Co-location Analysis of cDNAs from the transcriptome map and published QTLs revealed 147 co-located TDFs. We isolated, cloned and sequenced 38 TDFs for resistance QTLs. Sequence comparisons revealed some interesting homologies with known resistance genes such as chitinases, kinases or proteins which contain an NBS motif. In several cases also known response genes to stress reactions were detected.

Numerous primer combinations were applied for differential cDNA-AFLP for nematode (*Globodera pallida* Pa2/3) and *Phytophthora* resistance in leaves and tubers of a set of resistant and susceptible accessions, respectively. Between 20 and 50 differentially expressed bands were detected in each assay which in many cases were common to different accessions.

The average global response was five times higher in resistant accessions than in susceptible ones. For nematode infections between 30 and 75% of all differentially expressed TDFs were specific for roots. Sequence comparisons revealed several interesting homologies with known resistance genes or stress response genes in different species. In part known candidate genes such as 4-CL and a glucanase were re-detected with this technique.

Microarray analyses were performed in three accessions for nematode inoculations and in five accessions for *P. infestans* infections. In all cases we detected induced expression (>5 fold) after pathogen inoculation involving between 20 and 50 genes which can be assigned to different classes. Among them figure PRP proteins, NBS resistance genes, kinases, known R genes and several other genes with different functions. Although similar types of genes were detected in all cases, the particular individual genes generally differed between accessions. Also the particular response varied with respect to the frequency distribution of gene classes.

Mapping of known and detected candidate genes on the UHD population using appropriate primers for obtaining segregating polymorphisms revealed co-location with published QTLs in several cases.

With respect to QTL screening using linked SSR markers we have detected in progeny G so far three selectable QTLs on chromosomes III, VI and VII according to the significance values. Significant QT allele effects were observed on chromosome VI for parent P2 (GON) while this was the case for the other parent (JAM) at the other two locations. In progeny E we have detected four selectable QTLs located on chromosomes III, V, VI and X while in progeny D only one QTL was detected on chromosome VIII.

Discussion

TDF markers have generally a concrete biological function since they are derived from mRNA. In our studies we have detected several promising cDNAs with a relevant biological meaning which are co-located with published QTLs for disease resistance and could therefore represent this QTL.

Our results show that the differential cDNA-AFLP technique represents a powerful tool to discover known and novel resistance or response genes to pathogen infections since several TDFs were obtained which have a relevant biological function. Some of them have been identified previously by other techniques such as direct EST sequencing from subtracted libraries, showing the viability of our approach.

For the same reasons also micro array analysis represents a powerful tool to discover known and novel resistance or response genes. The sequences of differentially hybridised cDNAs are known and can be retrieved via TIGR *Solanum tuberosum* Gene Index (StGI) at http://www.tigr.org/tdb/tgi/stgi/. Appropriate primers can be designed to characterise and isolate the homologous cDNAs in different *Solanum* wild species. These can be used for downstream applications such as genetic transformation or allelic diversity studies in different germplasm. They can be mapped also to the UHD reference map in order to detect associations with QTLs.

In several cases we could integrate known and newly detected candidate genes into the UHD reference map and detected some associations with published QTLs. However, different linked genes could be the true responsible for the QTL effect. Therefore, at the very end it will be necessary to perform a complementation assay in order to verify this hypothesis. If a candidate gene should represent a false positive, then it can be used at least as allele-specific marker for marker assisted selection.

Published QTL for *P. infestans* resistance have been reported for all 12 potato chromosomes and the SSR map of Milbourne et al. (1998) allows to project linked SSR markers for all loci. We have screened published QTL positions for *P. infestans* resistance *via* closely linked SSR markers for the presence of QT allele differences in three different progenies. In this way we have established a "genotypic print" of each parent (QTA genotyping) indicating selectable QTL positions and the corresponding SSR markers for marker-assisted selection (MAS) in different genetic backgrounds. These markers can be applied within potato breeding programmes for all crosses which involve the corresponding parental genotypes.

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GISH AND FISH ANALYSES OF TETRAPLOID SPECIES OF THE SERIES *LONGIPEDICELLATA* AND *CONICIBACCATA*

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Of the approximately 200 potato species (genus *Solanum* section *Petota*), 36% are polyploids and 64% are diploids (2n=2x=24) (Hijmans et al., 2007). Determination of the type of polyploidy and the development of the genome concept for species of the section *Petota* has been based mainly on the analysis of chromosome pairing in species and their hybrids (Marks, 1955, 1965; Hawkes, 1958; Irikura, 1976; Ramanna and Hermsen, 1981; Matsubayashi, 1991; Lopez and Hawkes, 1991; Gavrilenko, 2007). Chromosome pairing relationships in interspecific hybrids and in polyploid species have been interpreted by genome formulae, although authors gave genomes different symbols. According to Matsubayashi (1991), all diploid potato species (2n=2x=24) comprise one major genomic group A and they differ only by cryptic structural differences. For example, *S. verrucosum* possess by the basic A genome (AA); diploid Mexican species of the series *Pinnatisecta* possess by the genome variant $A^{pi}A^{pi}$; diploid species of the series *Conicibaccata* possess by the genome variant $A^{c}A^{c}$. Wild tetraploid species (2n=4x=48) of the series *Longipedicellata* and *Conicibaccata* are considered as strict allopolyploids based on meiotic analyses. Genomic formula AABB was proposed for tetraploid species of series *Longipedicellata* and genomic formula $A^{c}A^{c}$C`C` - for tetraploid species of series *Conicibaccata* (Matsubayashi, 1991). Based on analysis of chromosome pairing in hybrids, diploid species *S. verrucosum* (AA genome) was considered as putative contributor of the common A genome of natural allopolyploids (Marks, 1965). A phylogenetic relationship of *S. verrucosum* and Mexican polyploid species was supported by similarity their plastid DNA (Spooner and Sytsma, 1992) and geographical and morphological data. The origin of the second component genomes of natural allopolyploids is being elucidated by current FISH, GISH (Pendinen et al., in press), and single-copy nuclear DNA markers (Spooner et al., in press).

The objective of this research was to study through fluorescent *in situ* hybridization techniques (GISH and FISH) the polyploid origin of Mexican tetraploid species of the series *Longipedicellata* (*S. stoloniferum* and *S. hjertingii*) and tetraploid species of the series *Conicibaccata* (*S. colombianum*). We chose the putative diploid species progenitors for tetraploid species based on prior hypotheses of classic genome analyses (Marks, 1965; Irikura, 1976) and the nuclear DNA sequencing phylogeny of Spooner et al. (in press). Three series of experiments were performed. (A) We probed mitotic and meiotic chromosomes of the tetraploid species of series *Longipedicellata* with DNA of their proposed diploid progenitors, (B) we hybridized mitotic chromosomes of tetraploid species of series *Conicibaccata* with DNA of their proposed diploid progenitors, and (C) some of polyploid and diploid species were analyzed by FISH using 5S and 45S rDNA probes.

Genomic DNAs were isolated from leaves of diploid species: *S. verrucosum* (PI 545745), *S. jamesii* (PI 458424), *S. cardiophyllum* (PI 595465), *S. ehrenbergii* (PI 275216), *S. santolallae* (PI 195168), *S. andreanum* (PI 320345), *S. violaceimarmoratum* (PI 473397), *S. pascoense* (PI 365339), and *S. piurae* (PI 310997). DNA of *S. verrucosum* was labeled with DIG-UTP. DNAs from the other diploid species were labeled with Biotin-UTP. DIG-labeled DNA was detected with rhodamine anti-DIG conjugate and biotin labeled probes were detected with FITC conjugated avidin. Mitotic chromosomes were prepared from tetraploid species: *S. stoloniferum* (PI 186544, PI 497998, PI 255546, PI 251740), *S. hjertingii* (PI 545713) and *S. colombianum* (PI 498150) for GISH and FISH analyses. Meiotic chromosomes were prepared from *S. stoloniferum*. 45S rDNA and 5S rDNA probes were used in comparative FISH analyses in different species. GISH and FISH analyses followed protocols of Jiang et al. (1996), Dong et al. (2001), Leitch et al. (1994).

(A) GISH was performed with mitotic chromosome preparations of tetraploid species of the series *Longipedicellata*: *S. stoloniferum* and *S. hjertingii* and differentially labeled DNA of diploid species in the following three series: (1) *S. jamesii* and *S. verrucosum*, (2) *S. cardiophyllum* and *S. verrucosum*, (3) *S. ehrenbergii* and *S. verrucosum* (Pendinen et al., in press). The results of GISH analysis showed clear parental genome discrimination in tetraploid *S. stoloniferum* and *S. hjertingii*, indicating that these species were derived from merging of two divergent parental genomes. Their 1st genome (-A) is highly homologous to the genome of *S. verrucosum* and their 2nd genome (-B) is homologous to the genome of Mexican diploid species of the series *Pinnatisecta* (*S. jamesii*, *S. cardiophyllum* and *S. ehrenbergii*). *Solanum stoloniferum* showed preferential bivalent pairing; intergenomic chromosome associations were not observed at diakinesis of *S. stoloniferum*. Our results showed that *S. stoloniferum* and *S. hjertingii* are strict allotetraploids (AABB) originated through merging two divergent genomes (A and B). The genome symbol B would denote genomes of Mexican diploid species of the series *Pinnatisecta* (2n=2x=24, BB) based on their homology to the second genome of the allotetraploid Mexican species *S. stoloniferum* and *S. hjertingii* (AABB) (Pendinen et al., in press).

(B) GISH was performed with mitotic chromosomes preparations of tetraploid species of the series *Conicibaccata* - *S. colombianum* and differentially labeled DNA of diploid species in the following six series: (1) *S. santolallae* and *S. verrucosum*, (2) *S. violaceimarmoratum* and *S. verrucosum*, (3) *S. santolallae* and *S. andreanum*, (4) *S. violaceimarmoratum* and *S. andreanum*, (5) *S. violaceimarmoratum* and *S. pascoense*, (6) *S. violaceimarmoratum* and *S. piurae*. GISH analysis of *S. colombianum* did not reveal differentiation of parental sub-genomes. This result was confirmed in several independent replications of each series of GISH experiments. In the first two series of GISH experiments (1,2) chromosomes of *S. colombianum* were hybridized with DNA of (1) *S. santolallae* or (2) *S. violaceimarmoratum*. We did not observe hybridization of DNA of *S. verrucosum* with chromosomes of *S. colombianum* that indicates on comparatively low level their homology. Red signal of fluorescence (DNA of *S. verrucosum* was labeled by rhodamin) was observed in high repetitive region of 45S rDNA of satellite chromosomes. Our result also indicate: (a) high level of homology between two component genomes of tetraploid species *S. colombianum* and (b) high homology between genome of *S. colombianum* and genomes of diploid species of the series *Conicibaccata* (*S. violaceimarmoratum)* and series *Piurana* (*S. andreanum, S. pascoense*).

(C) Diploid species (*S. verrucosum*, *S. jamesii*, *S. cardiophyllum, S. andreanum, S. violaceimarmoratum, S. pascoense*) have one terminally located 45S rDNA locus on the one chromosome pair and one 5S rDNA locus on another chromosome pair. *Solanum stoloniferum* has two chromosome pairs with one 45S rDNA locus and two another pairs of chromosomes with the 5S rDNA locus. Similar to the diploid species, the 5S rDNA locus of *S. stoloniferum* showed a relatively small size compared to the 45S locus. The two pairs of the 45S rDNA-bearing chromosomes of *S. stoloniferum* have different sizes of the 45S rDNA regions. In the sequential GISH and FISH experiments it was shown that one pair of chromosomes of the B subgenome of *S. stoloniferum* has the large 45S rDNA region. Whereas one pair of the A subgenome of *S. stoloniferum* has the small 45S rDNA regions. It is possible that the 45S rDNA regions of two subgenomes of *S. stoloniferum* were changed during coevolution of A and B genomes of this allotetraploid species (Pendinen et al., in press).

The genome of the tetraploid South-American species *S. colombianum* has two chromosome pairs with rDNA loci (one pair - with 45S rDNA site and one pair - with 5S rDNA site) and again – the 45S rDNA locus was terminally located and the 5S rDNA locus was located on the telomeric region of another chromosome pair. The 45S rDNA loci of *S. colombianum* is markedly bigger than the 5S rDNA loci. The 45S rDNA signals are distributed along almost the entire length of the satellite chromosome of *S. colombianum*.

This is the first study applying GISH analysis to investigate genome origins of natural polyploids within sect. *Petota*. From the practical point of view Mexican tetraploid species possess by a high level of disease and pest resistance; knowledge of their genome constitution will help breeders in introgressive programs to enrich genepool of common potato.

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CHARACTERIZATION OF POTATO TUBER SKIN AND HEAT INDUCED RUSSETING

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The potato (*Solanum tuberosum* L.) periderm is made up of three cell types: phellem, phellogen and phelloderm. The phellem, forms a series of layers at the outermost level of the periderm, and is derived from the underlying meristematic phellogen layer. Inward cell divisions of the phellogen give rise to the parenchyma-like phelloderm. As phellem cells develop, they become suberized and then die, forming a protective barrier that prevents pathogen invasion and fluid loss, and designated the 'skin'. To identify factors involved in phellem/skin development and that play a role in its defensive characteristics, two-dimensional gel electrophoresis (2-D) was used to compare the skin and parenchymatic flesh proteomes of young developing tubers. Proteins exhibiting differentially high signal intensity in the skin were sorted by functional categories. As expected, the skin proteome was enriched in proteins whose activity is characteristic of actively dividing tissues; however, 63% of the proteins were identified as involved in plant-defense responses to biotic and abiotic stresses. This group included three isozymes of caffeoyl-CoA *O*-methyltransferase and five isozymes of peroxidase that may play a role in suberization processes. The results shed light on early events in skin development and further expand the concept of the periderm as a protective tissue containing an array of plant-defense components.

Hot climate affects the appearance of potato tubers; the heat induces russeting of the skin, thus reducing the marketability of the crop. Histological studies indicated that in elevated temperatures the development of the periderm and suberization of the phellem layers are enhanced in comparison to control samples. Accordingly, it is suggested that the skin of potatoes grown under high temperatures is thick and non-flexible and tends to crack upon tuber expansion, resulting with netted/russeted appearance. In view of global warming and in order to improve skin quality of potatoes grown in hot climates we are attempting to identify the factors/genes involved in heat induced russeting, using microarray and 2-D approaches. Comparison of skin tissues isolated from tubers that were exposed to high soil temperatures and control tubers indicated of heat induction of ABA signaling and ethylene and IAA biosynthesis, an increase in heat shock proteins, and the involvement of up- and down-regulated transcription factors.

The results presented herein contribute to our understanding of periderm/skin development and its characteristics as a protective tissue against biotic and abiotic stresses.

INHERITANCE OF RESISTANCE OF SOLANUM NIGRUM TO PHYTOPHTHORA INFESTANS

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Summary

Two accessions of *Solanum nigrum* L were crossed, resistant and susceptible to *Phytophthora infestans* isolate MP 324. Their progeny were screened in detached leaf assay for resistance to *P. infestans*. Fifty individuals in F1 progeny were all resistant to this isolate. The segregation ratio of resistant to susceptible individuals of 378 F2 progenies was closest to 3 : 1. Out of 66 individuals of BC_1 progenies, obtained after crossing individual of F1 with susceptible parental parent, 26 were susceptible and 40 were resistant to *P. infestans*. Segregation patterns indicate monogenic dominant inheritance of resistance of *S. nigrum* acc. 984750019 to *P. infestans* isolate MP324.

Key words leaf assay; late blight; breeding

Introduction

Late blight, one of the most important diseases of potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esulentum* Mill.) is caused by oomycete *Phytophthora infestans* (Mont.) de Bary. Black nightshade (*Solanum nigrum* L.), a weed plant, has been regarded as a non-host for *P. infestans* for many years, due to the lack of infection in laboratory conditions (Colon et al., 1993; Platt, 1999), as well as scarce infection in natural conditions in England, the Netherlands and Wales (Hirst and Steadman, 1960; Flier et al., 2003; Deahl et al., 2004). However Flier et al. (2003) suggested a reconsideration of the present status of *S. nigrum* as a non-host plant to *P. infestans* based on the presence of field infections and results obtained in detached leaf inoculation studies. Studies by Colon et al. (1993), Flier et al., (2003) and Lebecka (2007) suggested *R* gene based resistance to *P. infestans* in *S. nigrum*, though the mechanisms of resistance to *P. infestans* in this species are not well known.

The goals of these studies were to advance the understanding of the mode of inheritance of resistance to *P. infestans* in *S. nigrum*.

Materials and methods

Plant material

Plant material used in this study originated from two accessions of *S. nigrum*: #13 (originated from seeds which were kindly provided by Agricultural University, Warsaw, Poland) and N19 (originated from seeds of accession *ngr* 984750019, kindly provided by Botanical and Experimental Garden of the Radboud University, Nijmegen, the Netherlands). Susceptible to *P. infestans* #13 and resistant to *P. infestans* N19 were selected for crossing based on results obtained in previous studies (Lebecka, 2007). Three experiments were performed:

I. Fifty individuals of F1 obtained after cross of #13 with N19, 35 siblings obtained after self-pollination of susceptible female parent #13 and 30 siblings obtained after self-pollination of resistant male parent N19. Three leaves per plant were evaluated. in one test.
II. 378 of unselected F2 plants of cross #13 x N19. Five leaves per plant were tested in each of three to 5 tests performed.
III. 66 of unselected BC1 plants obtained after a crossing of one F1 individual with susceptible parent #13. 10 leaves per plant were tested in each of three tests performed.

Phytophthora infestans isolate

P. infestans isolate MP 324 was used for testing resistance in detached leaf assay. Inoculum consisted of a sporangial suspension that was prepared as described by Zarzycka (2001) from sporulating lesions of potato leaflets and adjusted using haemocytometer to the concentration of 50 sporangia in μL^{-1}.

Detached leaf assay

Fully developed leaves were detached from the middle part of the 6 to12 week old greenhouse-grown plant. Leaves were placed on the wet cellulose wadding in a plastic tray. Each leaf was inoculated by depositing 30 µl of the inoculum on the abaxial side of the leaf. The trays with leaves were covered with glass. The inoculated leaves were incubated for 7 days at 16 $^{\circ}$C with a constant illumination of about 1600 lx. After the first 24 h of incubation the leaves were turned the abaxial side down. Each leaf was scored as resistant, when lacking the visible infection symptoms or possessing nonsporulating necroses of the size less or equal to the area of inoculation drop, and susceptible, with sporulating lesions.

Results

It was assumed that plants which had been used as parental forms in crossing are highly homozygous due to predominantly self-pollinating of *S. nigrum* species (Edmonds and Chweya, 1997).

Exp. I. To check out homozygosis of parental *S. nigrum* forms the evaluation of the reaction of progeny obtained after self-pollination of both parental forms to inoculation with *P. infestans* was performed. All 35 individuals from progeny of susceptible *S. nigrum* #13 were infected by *P. infestans* MP 324 isolate and all 30 individuals from progeny of resistant *S. nigrum* N19 were resistant to this isolate. All 50 individuals from F1 progeny obtained after crossing of *S. nigrum* #13 with *S. nigrum* N19 were resistant to inoculation by *P. infestans* MP 324 isolate.

Exp. II. The variation in reaction of leaves from the same plant was observed within and among tests. All the leaves were resistant in each of tests of 147 individuals, and for 112 individuals less than 0.25 leaves were infected, and these individuals were classified as resistant. 63 individuals were classified as susceptible, for which more than half of the leaves tested were infected. 28 clones were not classified to any of these classes, because the results of detached leaf assay were not consistent. The ratio is closest to 3:1 (Table 1).

Exp. III. Among 66 of BC1 progeny obtained after cross of F1 plant with susceptible *S. nigrum* #13, 26 individuals were susceptible and 40 were resistant to *P. infestans* MP324. The expected 1:1 ratio was analyzed by chi-square test and chi-square value = 2.97 (P = 0.085).

Discussion

Based on cytological studies by Krishna Rao (1971) it is concluded that the natural hexaploid of *S. nigrum* is an autoallohexaploid, which has three sets (genomes) of 12 chromosomes each, two of which are to a large extent homologous to each other. It was also observed that chromosome pairing appears to be restricted to bivalent formation and the absence of quadrivalent formation in the natural hexaploid was explained by its genetic suppression. Some other observations supports the hypothesis of lack of homology of between the genomes in *S. nigrum* (after Edmonds, 1979). Theoretical genotype, gametes and segregation ratios are presented in Table 2. Based on the results obtained in detached leaf assay the segregation pattern fit to the theoretical ratio of resistant to susceptible individuals 1 : 0 in F1, is closest to 1 : 1 ratio in progeny of BC1 to susceptible parent, and is closest to 3 : 1 in F2 progeny. Taking into consideration self pollinating of this species most feasible is that parental forms were homozygous for that gene, and two possible genotypes of resistant parent are proposed, A_1A_1aaaa – assuming that the plant is autoallohexaploid, and $A_1A_1a_2a_2a_3a_3$. - when it is allohexaploid. In both cases the inheritance of resistance may be monogenic dominant.

Table 1. Segregation ratios of resistance to Phytophthora infestans isolate MP 324 in F2 progeny of S. nigrum susceptible #13 x resistant acc. 984750019 cross evaluated in the detached leaf assay

Share of infected leaves	Resistant 0-0.25	Intermediate 0.25 - 0.5	Susceptible 0.5-1.0
Observed	259	56	63
Expected (3 : 1)	283		94

Table 2. Theoretical genotype, gametes and segregation pattern in self pollinating autoallohexaploid and allohexaploid resistant plant crossed to susceptible one

Genotype	Gamete	Theoretical ratio of R : S	
		F1	F2
Autoallohexaploid			
A_1A_1aaaa	A_1aa	**1 : 0**	**3 : 1**
a_1a_1AAaa	$a_1AA + 4\ a_1Aa + a_1aa$	5 : 1	
a_1a_1AAAA	a_1AA	1 : 0	35 : 1
A_1A_1AAAA	A_1AA	1 : 0	63 : 1
Allohexaploid			
$A_1A_1a_2a_2a_3a_3$	$A_1a_2a_3$	**1 : 0**	**3 : 1**
$A_1A_1A_2A_2a_3a_3$	$A_1A_2a_3$	1 : 0	15 : 1
$A_1A_1A_2A_2A_3A_3$	$A_1A_2A_3$	1 : 0	63 : 1

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INTROGRESSION OF VIRUS RESISTANCES FROM *SOLANUM ETUBEROSUM* INTO CULTIVATED POTATO AND THE IDENTIFICATION OF MOLECULAR MARKERS LINKED TO ITS POTATO LEAFROLL VIRUS (PLRV) RESISTANCE

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Potato virus Y (PVY) and potato leafroll virus (PLRV) are two of the most important viral pathogens of potato. Infection of potato by these viruses can result in substantial yield and quality loss in commercial potato production and in the rejection of seed in certification programs. Host plant resistance to these two viruses has been identified in *Solanum etuberosum* Lindl., a diploid (2n=2x=24) potato species endemic to Chile. Classified as 1EBN according to the endosperm balance number hypothesis (Johnston and Hanneman 1982), *S. etuberosum* cannot be readily sexually hybridized with diploid or tetraploid *S. tuberosum* L. subsp. *tuberosum*. Barriers to the introgression of virus resistances from *S. etuberosum* into cultivated potato were overcome by the use of somatic hybridization (Novy and Helgeson 1994a).

Tetraploid (2n=4x=48) somatic hybrids [*S. etuberosum* + (*S. tuberosum* x *S. berthaultii*)] were successfully hybridized with the potato varieties 'Katahdin' and 'Atlantic'. Somatic hybrid parents and 1st generation sexual progeny expressed PVY resistance derived from *S. etuberosum* (Novy and Helgeson 1994b). Subsequent evaluations demonstrated that both PVY and PLRV resistances derived from *S. etuberosum* were present in second generation progeny as well (Novy et al. 2002). In addition, resistance to green peach aphid, a primary vector of PVY and PLRV, was demonstrated in two generations of progeny derived from *S. etuberosum* (Novy et al. 2002).

Three generations of progeny derived from *S. etuberosum* were evaluated over multiple years in replicated field evaluations having high virus pressure and aphid populations. PVY and PLRV resistances of *S. etuberosum* were shown to be stably expressed in two generations of progeny. However, while PLRV resistance was transmitted and expressed in the third generation of backcrossing to cultivated potato, PVY resistance was lost (Novy et al. 2007). Segregation for PLRV resistance in a BC_3 population was skewed towards a resistant response and was best explained by a 1:1 segregation model with PLRV resistance conferred by a single, dominant gene. Evidence of a major gene for PLRV resistance confirms an earlier observation by Chavez et al. (1988) that PLRV resistance in *S. etuberosum* appeared to be under simple genetic control, with the mechanism of resistance being identified by the authors as resistance to PLRV titer buildup in foliage. Field and grafting studies conducted in our program support reduced PLRV accumulation in the foliage as contributing to the observed PLRV resistance. However, our research also has identified the inhibition of the systemic spread of PLRV from infected foliage to tubers as a major component of the observed PLRV resistance (Novy et al. 2007). We believe this mechanism of resistance is conferred by a single gene.

Molecular analyses also support a monogenic model for PLRV resistance, with localization of resistance to linkage group 4 of the potato/tomato linkage maps (Gillen and Novy 2007). Subsequent research has identified several PCR-based molecular markers linked to PLRV resistance, with applications for marker-assisted selection. Linkage analysis indicates PLRV resistance to be located at or beyond the end of linkage maps for chromosome 4 in potato and tomato. Several markers of tomato linkage group 4 that are distal and unlinked to the PLRV resistance show linkages with markers from chromosome 9. This data, in conjunction with a published linkage map of *S. etuberosum* (Perez et al. 1999), indicates a possible chromosomal rearrangement of chromosome 4 of *S. etuberosum*.

The PLRV resistance contributed by *S. etuberosum* appears to be an example of a monogenic trait that has shown stable transmission and expression through three sexual generations derived from the original somatic hybrid; these characteristics make this resistance useful for developing PLRV resistant potato cultivars. Our efforts in developing molecular markers for marker-assisted selection would further facilitate the use of this PLRV resistance in potato breeding programs.

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CHARACTERISATION AND PYRAMIDING OF LATE BLIGHT *R* GENES FROM WILD SOLANUM SPECIES

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Abstract

The distinction between field resistance and resistance based on *R*-genes has been proven valid for many plant pathogen interactions. This distinction does not seem to be valid for the interaction between potato and late blight. A locus involved in late blight resistance derived from *S. microdontum*, will be presented. The results provides additional evidence for this lack of distinction. The resistance is associated with a hypersensitive response and results in a delay of infection of about 1-2 weeks. Both a quantitative as well as a qualitative genetic approach were used, based on data from a field assay. QTL analysis identified a QTL on chromosome 4 after correction of the resistance data for plant maturity. A qualitative genetic analysis resulted in the positioning of this locus on the short arm of chromosome 4 in between AFLP marker pCTmACG_310 and CAPS markers TG339 and T0703. This position coincides with a conserved *Phytophthora* *R*-gene cluster which includes *R2, R2-like, RPi-blb3* and *RPi-abpt*. This implies that *RPi-mcd1* is the fifth R-gene of this NBS-LRR cluster. The implications of our results on *R*-gene based and field resistance are discussed.

Pyramiding of R-genes can be a solution to increase both durability and level of resistance. The resistance gene, $R_{Pi\text{-}mcd1}$ was combined with $R_{Pi\text{-}ber}$, introgressed from *S. berthaultii.* This resulted in a segregating diploid *S. tuberosum* population. Individual genotypes from this segregation population were classified into four groups, carrying no *R*-gene, with only $R_{Pi\text{-}mcd1}$, with only $R_{Pi\text{-}ber}$, and a group with the pyramided $R_{Pi\text{-}mcd1}$ and $R_{Pi\text{-}ber}$ by means of flanking molecular markers. The levels of resistance between the groups were compared in a field experiment in 2007. The group with $R_{Pi\text{-}mcd1}$ showed a significant delay to reach 50% infection of the leaf area of three days. The group with $R_{Pi\text{-}ber}$ showed a delay of three weeks. The resistance level in the pyramid group suggested an additive effect of $R_{Pi\text{-}mcd1}$ with $R_{Pi\text{-}ber}$. This suggests that potato breeding can benefit from combining individual *R*-genes, irrespective of the weak effect of $R_{Pi\text{-}mcd1}$ or the strong effect of $R_{Pi\text{-}ber}$.

ABIOTIC STRESS RESPONSES IN POTATO: GENE EXPRESSION AND BEYOND

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A growing plant has to face a number of challenges throughout its life, and environmental stresses, also called abiotic stresses, have an important impact on plant survival and productivity. In potato, abiotic stresses can have dramatic consequences, ranging from a mild reduction in yield to a premature death of the plant.

We have investigated the response of potato genotypes to three different stresses: cold, salinity and drought. For each stress, between one and three potato genotypes belonging to different *Solanum tuberosum* groups (including native Andean landraces and one *phureja* genotype) have been used. Each genotype has been studied at different time points for each stress respectively, using a cDNA microarray approach.

The main responses to cold non-freezing temperatures included (i) the up-regulation of genes of simple sugars synthesis, (ii) the up-regulation of key enzymes belonging to the polyamines pathway, and (iii) the strong down-regulation of photosystem-related genes.

Salt stress also induced a down-regulation of photosynthesis. That might be the cause of the observed mild repression of lipid, carbohydrate and amino acid metabolism genes. On the contrary, genes promoting protein folding and plant defence mechanisms were generally up-regulated.

Drought stress was characterized by the same kind of up-regulation of protein folding genes, but also by an up-regulation of genes for small osmoprotectant molecules (sucrose, proline, small polyamines). The photosystem was down-regulated: this seemed to be a feature shared by all three stresses.

Additional research was conducted to corroborate the microarray results. If the real-time RT-PCR results were largely in accordance, proteomics and even more metabolomics results were less clear-cut. They rather confirmed that there was still a gap between a gene’s expression, its protein accumulation and the corresponding metabolite’s abundance.

TRANSCRIPTIONAL PROFILING IN POTATO: IN SEARCH OF CANDIDATE GENES UNDERLYING QTLS FOR TUBER QUALITY

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The identification and cloning of genes that underlie major QTLs in crop plants is a difficult challenge that generally involves fine mapping and map-based cloning approaches. Differential gene expression between individuals of a segregating diploid potato population (C x E) can be screened for association with a targeted tuber quality trait. Transcript profiling using a bulked segregant analysis (BSA) approach, targeting extreme phenotypes from within the population, can quickly reveal potential candidate genes. In such an approach identified genes are then screened on the individual genotypes using qRT-PCR to confirm the association of gene expression with the trait of interest through eQTL analysis. In order to reduce the number of contrasting bulks that need to be designed for the different quality traits, we have performed hybridizations of a number of individuals (98) from the same population thereby circumventing the need for qRT-PCR and allowing *in silico* bulking of additional traits. Promising candidate genes that exhibit strong association (eQTL) with the trait of interest are then converted into genetic markers and positioned on a genetic map. This approach allows for an un-biased search for genes associated and genetically linked to the QTL of interest. Furthermore, this data set allows for the analysis of individual genes belonging to a relevant pathway involved in controlling tuber quality traits and can aid in the identification of important gene family members.

We have tested this approach on different tuber quality traits including flesh colour, texture after cooking, chip colour and plant maturity, and have identified a number of candidate genes that map under the QTL of interest and are the focus of further research.

EFFECTOR GENOMICS AND CISGENIC EXPLOITATION OF LATE BLIGHT R GENES FROM WILD SOLANUM SPECIES; THE KEYS TO DURABLE RESISTANCE STRATEGIES IN POTATO

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Potato is the world's fourth largest food crop yet it continues to endure late blight (LB), a devastating disease caused by the Irish famine pathogen *Phytophthora infestans*. Exploiting genetic resistance is the best disease management strategy but current approaches have been unsuccessful. The rapid break down of the first introgressed resistance genes to *P. infestans* (*Rpi*) from *Solanum demissum* stimulated breeders to reconsider their breeding goals and subsequently, efforts towards improving LB resistance were focused on increasing partial resistance by using race-nonspecific sources of resistance. However, under long day conditions, breeders using this strategy have achieved little progress, the major draw-back being the strong linkage between foliage resistance and late foliage maturity. We therefore anticipate that breeding for LB resistance in potato, aiming at substantially contributing to disease management, requires, by one way or another, the deployment of *Rpi* genes. In an attempt to identify novel *Rpi* genes and to gain insight into *Rpi* gene diversity we have carried out an extensive germplasm screen and are currently mapping *Rpi* loci with the ultimate goal to clone complementary *Rpi* genes. To date, a total of 13 *Rpi* genes have been isolated from a number of different species. All belong to the NB-LRR class of *R* genes and are members of homologous gene clusters of varying sizes. As more and more *Rpi* genes are identified and cloned, the chance increases that new *Rpi* gene alleles reside at known and well-characterized loci, enabling the use of comparative genomics, and thus the development of efficient allele mining strategies. Moreover ongoing potato and tomato genome sequencing projects by international consortia are providing a (complete) survey of the distribution of *R* gene clusters in the *Solanaceae,* enabling even faster cloning of *Rpi* genes. Challenges that remain are how to predict *Rpi* gene durability and how to introduce durable combinations of *Rpi* genes into existing and future varieties in the most efficient and sustainable manner. We believe that knowledge of effector diversity will inform likely durability of *Rpi* genes. We have pioneered the exploitation of putative effector genes predicted computationally from the *P. infestans* genome to accelerate the identification, functional characterization, and cloning of *Rpi* genes. An initial set of 54 effectors containing a signal peptide and an RXLR motif were profiled for activation of innate immunity (avirulence or Avr activity) on wild *Solanum* species using the PVX expression system. Many tentative *Avr* candidates were identified, including the RXLR effector family IpiO, which induced HRs in *S. stoloniferum, S. papita* and the less-related *S. bulbocastanum,* the source of *Rpi-blb1*. Genetic studies with *S. stoloniferum* showed co-segregation of resistance to *P. infestans* and response to IpiO. Transient co-expression of *IpiO* with *Rpi-blb1* in a heterologous *Nicotiana benthamiana* system identified *IpiO* as *Avr-blb1*. A candidate gene approach lead to the rapid cloning of the functionally equivalent *Rpi-blb1* homologues *Rpi-sto1* and *Rpi-pta1* from *S. stoloniferum* and *S. papita* respectively. These findings indicate that effectoromics enables discovery and functional profiling of late blight *Rpi* genes at an unprecedented rate and promises to accelerate the engineering of late blight resistant potato varieties in the future.

The traditional way of introducing resistance is introgression breeding by inter-specific crosses and repeated backcrosses with cultivated potato, which is a very slow and inefficient process. The result is introgression of a piece of DNA with the gene of interest, surrounded by other alien genes of the donor species. This is called linkage drag and is almost always connected with linked genes coding for traits with a negative impact, such as increased glycoalkaloid content. Furthermore, since potato is a heterozygous tetraploid, few out-crossed progeny retain all of the desirable parental traits, and much pre-breeding is required to remove the most important linked genes coding for negative traits. It can be said that introgression breeding is a multiple step approach resulting in the introduction of a target gene, e.g. an *Rpi* gene, with linkage drag. For durable LB resistance strategies, efficient stacking of *Rpi* genes from one or several species is essential, but in practice this will enlarge the linkage drag problems considerably. Their introduction by genetic modification (GM) is a much more efficient way to improve resistance in one step and in a short period. It can even be applied to existing varieties with a long history of safe use. Currently, *Rpi* genes of natural origin, so called cisgenes, can be introduced using marker-free transformation systems leading to cisgenic plants with only the gene(s) of interest and without linkage drag.

GENOTYPING AND PHENOTYPING OF *SOLANUM TUBEROSUM* (+) *S. BULBOCASTANUM* SOMATIC HYBRIDS AND THEIR ANTHER-DERIVED HAPLOIDS

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Somatic hybridization *via* protoplast fusion provides a powerful tool to overcome EBN crossing barriers in potato (4x=48, 4EBN), allowing the integration of parental nuclear and cytoplasmic genomes. We used protoplast fusion to overcome incompatibility between the 2x(1EBN) *Solanum bulbocastanum* and 2x(2EBN) *S. tuberosum* haploids (Savarese et al., 2005). In this paper we report on the genotyping and phenotyping of 4x and 6x somatic hybrids previously developed (Savarese et al., 2005), and on the production of androgenetic haploids from these somatic hybrids.

For genotyping, the cytoplasmic genome of the somatic hybrids was analyzed with 2 gene-specific primers for cpDNA and 2 for mtDNA that detected polymorphisms between the fusion parents. Most somatic hybrids inherited the plastidial and mitochondrial DNA of the cultivated parent (Table 1, Figure 1). However, a few hybrids with novel cytoplasmic genome were also identified. Three of them inherited the cpDNA from the wild parent and the mtDNA from the cultivated one. Other two hybrids had a rearranged mitochondrial genome, with fragments derived from both parents.

Table 1 – Synthesis of results from cpDNA and mtDNA analysis of 11 S. tuberosum (+) S. bulbocastanum somatic hybrids. tbr=S. tuberosum DNA type; blb=S. bulbocastanum DNA type.

Somatic hybrid	cpDNA	mtDNA
6A*, 6E*, 6G*, 9M*, 9B**, 9AP*	tbr	tbr
5A*, 9I*, 9R**	blb	tbr
9AI**, AQ*	tbr	tbr/blb§

*2n=4x; **2n=6x

§ re-arranged chondriome containing fragments of both parents

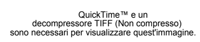

Figure 1 – PCR analysis of two parental genotypes and their somatic hybrids using the chloroplast (cp) DNA primer ALc1/3. M, DNA size marker; blb=*S. bulbocastanum;* tbr=*S. tuberosum*.

Genomic in situ hybridication (GISH) was an excellent tool to investigate the genomic dosage (cultivated *versus* wild) of somatic hybrids with different plody (Iovene et al., 2007). GISH confirmed the expected genomic ratio (*S. tuberosum* haploid:wild species) of 2:2 in 4x hybrids, and it revealed different chromosome constitutions among the three hexaploid hybrids obtained. Two of them had a predominance of *S. tuberosum* chromosomes and a genomic dosage of 4:2, the opposite was found in another hexaploid hybrid. These findings suggested that, if chromosome elimination and substitution are ruled out, hybrids with diverse EBN not normally obtainable via sexual reproduction can be produced.

Since very little is known on the glycoalkaloids of *S. bulbocastanum*, we made a preliminary investigation on the glycoalkaloids of our somatic hybrids. Hybrids inherited *S. tuberosum* glycoalkaloids, (solanine and chaconine) and other metabolites labelled 1, 2, 3 and 4 from *S. bulbocastanum*, From preliminary chemical and spectroscopic investigations they proved to be glycoalkaloids. In a few hybrids we also detected glycoalkaloids lacking in the parental genotypes. The structure and quantity of the unknown glycoalkaloids isolated will be further investigated. All *S.*

tuberosum (+) *S. bulbocastanum* hybrids were sterile in crosses with *S. tuberosum* genotypes. To overcome sterility problems, we performed *in vitro* anther culture (Rokka et al., 1995) of a sample of somatic hybrids and characterized haploids produced. Anther-derived embryos and green shoots were obtained from all tetraploid hybrids tested. Cytological analysis confirmed that the shoots developed were haploids, with a 24-chromosome complement. By contrast, the hexaploid hybrid used did not undergo gametic embryogenesis (Table 2).

Table 2 – Results from in vitro anther culture of five tetraploid and one hexaploid S. tuberosum (+) S. bulbocastanum somatic hybrids.

Somatic hybrid	Cultured anthers, no.	Embryos formed, no.	Shoots formed, no. (%)
5A (2n=4x)	100	90	84 (93)
6A (2n=4x)	440	46	36 (78)
6E (2n=4x)	200	1	1 (100)
9I (2n=4x)	740	4	4 (100)
9AP (2n=4x)	1000	6	5 (83)
9R (2n=6x)	200	0	-

Genotyping of haploids revealed a very low rate of polymorphisms for the cpDNA and mtDNA. By contrast, high degree of polymorphisms was detected at nuclear DNA level with ISSR primers. ISSR markers highlighted two categories of changes: loss of parental fragments and gain of novel fragments. The loss of parental fragments was the most frequent event observed. Phenotyping revealed a high level of variability in terms of tuber characteristics, plant habit and flowering. High variability was also detected on the glycoalkaloid synthesis. Indeed, some haploids either lost the ability to synthesise the parental glycoalkaloids or produced new ones. Research is in progress to study the fertility of haploids produced. The potential of using haploids from somatic hybrids to overcome the bottleneck of hybrid sterility and for basic research will be discussed.

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THE EXPRESSION OF Y-VIRUS RESISTANCE IN TRANSGENIC POTATO WITH COAT PROTEIN PVY GENE

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Abstract

The expression of target trait (PVY resistance) in transgenic potato plants with CP PVY gene of forth gene-engineered constructs was examined during 11 years in the field. It was found that strong infection pressure (artificial dual-year virus infection and plants-infectors using) promotes decreasing of incubation period of virus disease development and the elimination of susceptible samples in time of third year assessment. Seven transgenic PVY resistance sources with different types of virus resistance were defined. Moreover selected transgenic sources had a stable expression of the target trait.

Introduction

Gene technology is giving rise to a whole new set of possibilities in plant breeding. This new technique allows us not only to speed up the rate of acquiring the desirable traits expressed within a species, but also opens up the possibility of combining traits of totally different origins. The risks to connect with introduction transgenic plants in the field and gene silencing phenomena are considered the main reasons limited transgenic plant using. The gene silencing is an undesirable effect of the commercial potato production, but the studies, to direct on selection transgenic lines with stable target trait expression in field, exist a little in available literature. This article is dedicated to the results of field tests of transgenic potato with coat protein PVY gene (CP PVY gene) that were conducted over 11 years.

Material and Methods

Transgenic potato lines of cv. Belorusky 3 (B3) with coat protein (CP) gene of potato virus Y (PVY) strain N were obtained in Moscow Bioengeneering Centre by agrobacterial transformation of internodal stem segments with 4 cassettes of expression: A – p35SPLYCP (8 lines); B - p35SPLYCPdelATG (8 lines); C – p35SX28YCP (7 lines); D - p35SX28YCPdelATG (10 lines). They expressed foreign RNA and (or) protein [1]. The shoot regeneration was induced under the conditions of minimal development of callus on the selective medium with kanamycin. Transgenic potato line Bin without target gene was obtained by transformation of cv. Belorusky 3 and used as a control variant. Transgenic potato lines and were transferred for us within the framework of agreement on scientific cooperation.

Experimental scheme:

1st year – Transgenic potato line were propagated *in vitro* conditions and were took in greenhouse. Plants of non-transformed cv. B3, transgenic line Bin without target gene and transgenic lines with CP PVY gene were infected by mechanical inoculation of PVY. The visual virus symptom diagnostics was organized and the accumulation of PVY was identified by DAS-ELISA method.

2nd year – The tubers of healthy and weakly infected plants were planted in field. The potato plants were inoculated with PVY additionally. There was additional infected pressure by plants-infectors planting in field.

3d – 11th year -Vegetative progeny of PVY infected plants was grown on infectious background in field.

6th and 7th years – PVY resistant transgenic potato lines were tested by grafting and indicator-plant method.

Annually the PVY accumulation in the systemic leaves was detected by DAS-ELISA during flowering of potato plants. The various ELISA-kits contained the different enzymes were used. Thus ELISA-testing results are unified and presented as the average deviation (AD) of optical absorption value from significance threshold of positive data (P):

$$P = x + 3E,$$

where: x – the mean value of negative commercial control (wavelength A_{450} or A_{490}), E – the maximal negative control divergence from the average one.

The visual symptoms and PVY accumulation level were compared for each potato experimental plant. Next the following scale was dawn up:

1) AD < P – healthy plants, visual symptoms are absented,
2) P<AD<0.05 – weakly infected plants, visual symptoms are absented,
3) 0.051<AD<0.1 – middle infected plants, visual symptoms are indistinct or absented,
4) 0.101<AD – severe infected plants with distinct mosaic and necrotic symptoms.

Results and Discussion

The initial cv. Belorusky 3 has hypersensitivity and relative resistance to PVY. Most plants of non-transformed B3 and transgenic line Bin (without CP PVY gene) were unaffected with PVY in the first year (figure 1, 1st year) but they were heavy affected by Y-virus on the 5-6th year experiments. It is noted that the tubers of initial B3 lost the ability to germination after mechanical inoculation for two years running. According data the PVY resistance of transgenic potato line Bin was determined by PVY resistance of initial cultivar.

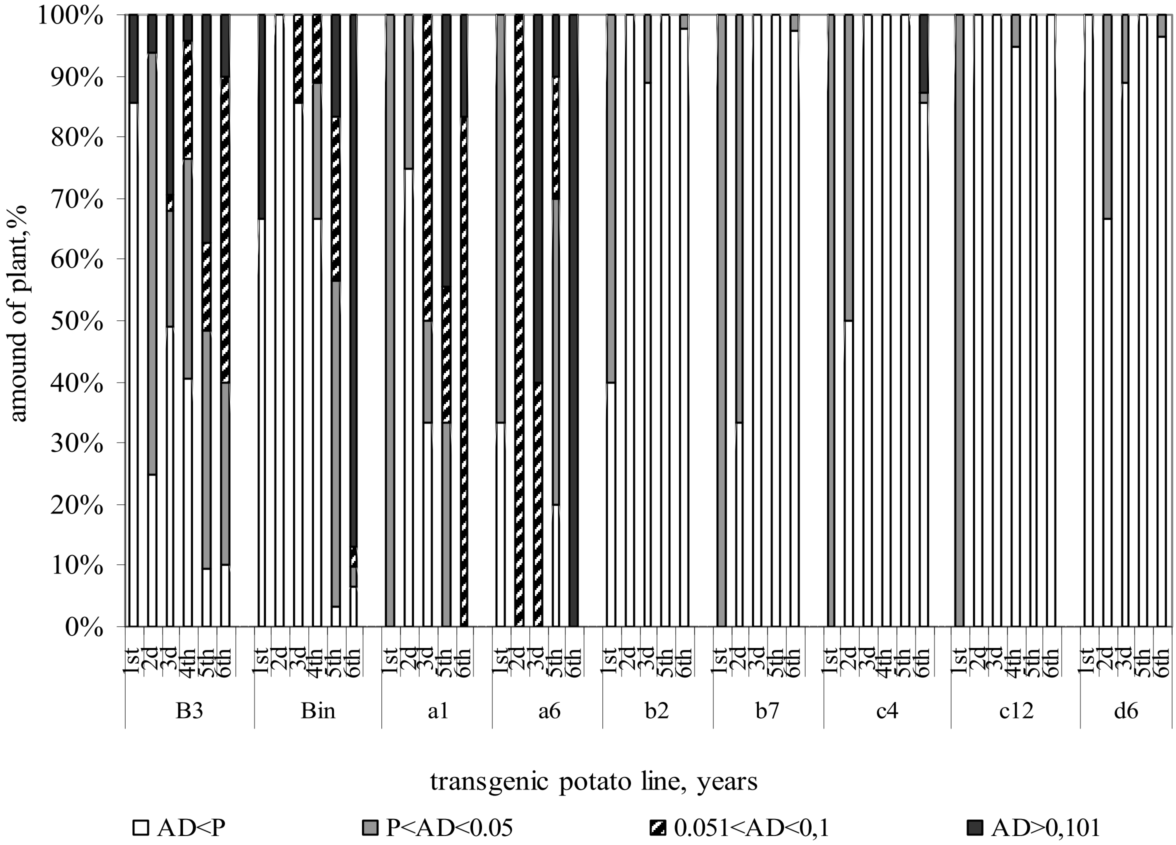

Figure 1. PVY accumulation in vegetative progeny of initial cv. Belorusky 3, transgenic potato line without CP PVY gene, and transgenic potato lines with CP PVY gene (the lines a1, a6 are PVY sensitive; the lines b2, b7, c4, c12, d6 are PVY resistant).

Notes: lines B3 and Bin are one-year PVY infected; transgenic lines a1, a6, b2, b7, c4, c12, d6 are dual-year PVY infected.

On first year the most of transgenic plants with CP PVY gene had positive results by ELISA, but most AD values didn't exceed 0.05 optical units. Transgenic potato lines of all four engineering constructions displayed the positive response on the primary pathogen introducing. On second year we observed "recovery" of vegetative progeny of annual PVY infected transgenic plants. The dual-year PVY inoculation of transgenic plants has no effect on the amount of virus affected plants in the resistant lines (figure 1, lines b2, b7, c4, c12). Increasing of infected pressure conduced to the division of transgenic lines on sensitive and resistant. Vegetative progeny of the dual-year infected transgenic plants traced 11 years: individual clones during six years, long-term propagation in according seed scheme – five years. Analysis of the gotten data showed that the sensitive transgenic lines with CP PVY gene could detected on third year. There were selected seven transgenic sources of the resistance to PVY among 33 transgenic potato lines: two transgenic lines b2 and b7 with engineering construction “B”; four lines c1, c3, c4, c12 with engineering construction “C”; one line d6 with engineering construction “D”. Plants of these lines were healthy after the long-term propagation for 11 years in field. Transgenic sources were testing by indicator-plant method and grafting (table 1, 2).

Table 1. PVY accumulation in tobacco plants cv. Samsun inoculated by the sap of the field transgenic plants (ELISA results)

Variants	AD values of the indicator tobacco plants, units of optical density				
	1	2	3	4	5
Negative control	-0,027	-0,025	-0,027	-0,028	-0,026
Positive control	**1,565**	**1,507**	**1,011**	**1,100**	**1,328**
Belorusky 3	**0,861**	**0,536**	**0,865**	-	-
b 2	-0,035	-0,034	-0,039	-0,035	-0,029
b 7	-0,034	-0,038	-0,027	-0,027	-0,031
c 1	-0,015	-0,014	-0,014	-0,008	-0,009
c 3	-0,009	-0,009	-0,013	-0,010	-0,015
c 4	-0,013	-0,011	-0,006	-0,013	-0,009
c 12	-0,015	-0,013	-0,010	-0,008	-0,011
d6	-0.027	-0.023	-0.024	-0.030	-0.028

Note: Negative control – the tobacco plants, inoculated by water; positive control – the tobacco plants, infected PVY strains; the significant positive values of ELISA-test are framed.

Table 2 The results of transgenic line testing by grafting method (30-35 days, the symptoms and AD value)

Transgenic line	Virus strain	Tomato plant		Graft	
		symptoms	AD value*	symptoms	AD value*
b 2	Y^O	rugose mosaic	***0,191*** ***0,497***	а) chlorosis б) graft necrosis	from –0,032 to -0,007
	Y^N	rugose mosaic bumpy lamina	***0,340*** ***0,199***	а) chlorosis б) necrotic symptoms в) necrotic symptoms	from –0,018 to -0,029
b 7	Y^O	bumpy lamina, rugose mosaic	***0,306*** ***0,285***	without symptoms	from –0,035 to -0,027
	Y^N	rugose mosaic	***0,868***	without symptoms	from –0,026 to -0,009
c 1	$Y^O + Y^N$	rugose mosaic	***0,470***	а) necrotic symptoms, б) potato rugose mosaic	from ***0,063*** to ***0,107***
	Y^{NTN}	rugose mosaic	***0,042***	а) necrotic symptoms, б) chlorosis	from –0,012 to -0,009
c 3	$Y^O + Y^N$	rugose mosaic	***0,038***	а) without symptoms б) necrotic symptoms	from –0,012 to –0,010
	Y^{NTN}	rugose mosaic	-	graft necrosis	-
c 4	$Y^O + Y^N$	rugose mosaic	***0,724***	graft necrosis	-
	Y^{NTN}	rugose mosaic	-	graft necrosis	-
c 12	$Y^O + Y^N$	rugose mosaic	***0,151***	without symptoms	from –0,013 to –0,008
	Y^{NTN}	rugose mosaic	***0,230*** ***0,387***	without symptoms	from –0,015 to –0,009
d6	Y^O	rugose mosaic	**0.307** **0.417**	а) without symptoms б) chlorosis	from –0,017 to ***0.013***
	Y^N	rugose mosaic	**0.257** **0.417**	а) without symptoms б) necrotic symptoms	from –0,006 to –0,003

*Note; * - the ELISA-test results; **0,063** – the significant positive values of ELISA-test are dedicated*

The plants of tobacco cv. Samsun were used as indicators of PVY latent infection. Tobacco plants were inoculated of sap of field transgenic plants and initial cv. Belorusky 3 (5 tobacco plants for each transgenic variant and 3 plants for initial cultivar). PVY accumulation in tobacco indicator plants was tested by ELISA (table 1).

The indicator tobacco plants inoculated with the sap of cv. Belorusky 3 field plants had mosaic symptoms according results of visual diagnostics and high level of PVY accumulation (AD values from 0.536 to 0.861, table 1). The indicator tobacco plants inoculated of the sap of transgenic plants were visually healthy and had the negative values of AD by ELISA-testing. Therefore the healthy transgenic potato plants with CP PVY gene, that were grown under infect pressure in field, not contained the PVY latent infection.

Tomato plants cv. Nevsky were used as plant stocks in grafting and were infected with the strain mix Y^{O} + Y^{N} and the stains Y^{NTN}, Y^{O}, Y^{N} in separation. In accordance with results transgenic potato lines showed different resistance to PVY. The grafts of transgenic lines b7, c12 were asymptomatic. Transgenic lines b2, c3 d6 responded the necrotic reaction on PVY attack. Transgenic line c4 had an extreme hypersensitivity to Y-virus. Systemic mosaic symptoms and positive AD values were noted for the transgenic line c1 infected ordinary strain of PVY (table 2). All transgenic lines with CP PVY gene were resistant to necrotic strain Y^{NTN} and Y^{N}.

The summation of the results of three tests (the artificial PVY inoculation, testing by indicator-plant method and grafting) has allowed defining the transgenic potato lines with CP PVY gene b7 and c12 as immunity to PVY.

Conclusion

Revealing target trait is changed in transgenic potato plants of cv. Belorusky 3 with CP PVY gene in direction as its improvement and worsening in comparison with initial cultivar. The determination of character change of target trait is possible for three years on condition of creation heavy selective pressure. The special approach is required for making the conditions of adequate selection to resistance. We used two-year inoculation by PVY in contrast to generally accepted one-year inoculation.

The sources of resistance to PVY have been selected between transgenic lines cv. Belorusky 3. Most of clones of seven selected transgenic sources of resistance to PVY were healthy according to ELISA test during long-term vegetative propagation in conditions of artificial infection background after two-year Y-virus inoculation. Transgenic potato lines with CP PVY gene showed different resistance to PVY. Among seven transgenic sources of resistance to PVY two transgenic lines b7 c12 were immunity to PVY.

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COMBINING MARKER ASSISTED SELECTION (MAS) AND SOMATIC HYBRIDIZATION FOR BETTER INTROGRESSION OF RESISTANCE GENES INTO CULTIVATED POTATO

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Plant somatic hybridization by protoplast fusion, mainly electrofusion, proved to be an efficient way to generate pre-breeding material in cultivated potato, which exhibited resistance to diseases (late blight, *Erwinia* sp., viruses: X, Y etc.) or pests (potato Colorado beetle) (Millam et al., 1995; Thieme et al., 2008). Although, the resistance genes may prove stable in BC1 and BC2 clones, the main problem with somatic hybrid material is that many back-crossings are required for the introgression of stable genes into potato gene pool, genes involved in conferring quantitative traits. For better and faster introgression a combination of the new strategies of cellular and molecular genetics are proposed in this paper.

The goal of our studies was to transfer mainly the resistance to late blight and/or potato Colorado beetle into cultivated well- characterized cultivars of potato from wild sexually incompatible *S. bulbocastanum* and the partially compatible *S. chacoense,* genotypes well characterized for resistance quantitative traits. For faster and better introgression of resistance genes into potato cultivars a combination of novel strategies such as using protoplasts of DNA mismatch repair (MMR) deficient plants and/or molecular markers tightly liked to leptines biosynthesis, glycoalkaloids known as deterrents of Colorado potato beetles, are to be presented. Many somatic hybrids were produced between the most responsive cv. Delikat and *S. chacoense* characterized for highest leptine content (PI 458310, Sturgeon Bay, USA). The analysis of ploidy level by flow cytometry, molecular analysis of RAPD markers linked with leptines, the leptine content of the somatic hybrids analysed by HPLC and laboratory feeding tests were used to characterize the valuable resistant somatic hybrid material. As for *S. bulbocastanum* GLKS 88.21(JKI, Germany), a genotype highly resistant to late blight has been used as a partner to different potato cvs. The putative somatic hybrids proved to be asymmetric by cytophotometric analysis, chromosome counts using DAPI staining and GISH (genome *in situ* hybridization). *S. bulbocastanum* proved very recalcitrant to *in vitro* culture and *Agrobacterium tumefaciens*-mediated transformation with msh2 genes (antisens – AS; negative competitive mutant – Apa). Consequently, the potato partner cv. Delikat with MMR deficiency were produced to be used in somatic hybridization experiments with S. bulbocastanum. The results show that a combination of novel strategies, which combine cellular and molecular techniques accounts for a faster and better introgression of quantitative resistance traits into cultivated potato.

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Genetic resources

RESISTANCE TO POTATO VIRUS Y IN THE WILD POTATO SPECIES SOLANUN TARNII, INTERSPECIFIC SOMATIC HYBRIDS, THEIR PROGENY AND ANALYSIS OF FEEDING BEHAVIOUR OF VIRUS TRANSMITTING GREEN PEACH APHID, MYZUS PERSICAE

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A major problem for seed potato growers is the quantity of insecticide they have to apply to obtain virus-free tubers and control pests, e.g. aphids, Colorado potato beetle (CPB) etc. Improving the resistance of potato to both viruses and pests could result in a marked decrease in insecticide application. This stimulated the search for linked resistance. There is evidence that the wild species *Solanum tarnii* could be an important source of resistance to pests and diseases. *S. tarnii* is a diploid wild tuber-bearing species (2n=2x=24) with lanceolate leaflets, belonging to the series *Pinnatisecta* section *Petota* (SPOONER et al. 2004). It occurs in Mexico and seeds were used to establish accessions of different germplasm collections. In our study the accession GLKS 2870 from Genebank External Branch 'North', Gross Lüsewitz, of the Leibniz Institute of Plant Genetics and Crop Plant Research, Germany was used, in order to characterize resistance traits to different pathogens and pests. Own studies showed that plants of *S. tarnii* are extremely resistant to *Potato virus* Y (PVY), high resistant to foliage blight caused by *Phytophthora infestans* (THIEME et al. 2008) and larvae of CPB suffered a high mortality when fed on the leaves of this species in laboratory trials (THIEME & THIEME 2006). Therefore, this wild species could be an important source of exotic genes for increasing the genetic diversity available to potato breeders. Because *S. tarnii* is reproductively isolated from tetraploid *S. tuberosum* it is difficult to cross with potato, and sexual or somatic hybrids between *S. tarnii* and common potato are unknown (JACKSON & HANNEMAN 1999). Protoplast fusion between the sexually incompatible species *S. tarnii* and cultivated potato cv. Delikat was used to produce somatic hybrids. Then using sexual crosses with potato varieties BC progenies were produced.

The interspecific somatic hybrids between *S. tarnii* and cv. Delikat, showed no symptoms of viral infection after mechanical inoculation in a greenhouse with six isolates of the most important virus strains (Tab. 1) and growing in the field (THIEME et al. 2008). This indicates that PVY resistance can be transferred from sexually incompatible wild species into cultivated potatoes by somatic hybridization. After backcrossing somatic hybrids with the susceptible cv. Delikat, all tested BC_1 lines expressed no PVY incidence when exposed to different strains of this virus. For BC_2 lines resistant and susceptible genotypes were identified (Tab. 1). The usefulness of this resistant plant material, its further molecular characterization and exploitation in potato pre-breeding programmes using combinatorial resistance, are discussed.

Aphids are the most important vectors of more than 50 viruses of potato, which cause serious losses in potato yield in the field. Whereas *Potato leaf roll virus* (PLRV) is transmitted by potato colonising aphid species, transmission of PVY is by non-potato colonizing aphids, such as the birdcherry oat aphid *Rhopalosiphum padi* in Northern Germany (THIEME et al. 1998).

The analysis of feeding behaviour was done using the green peach aphid, *Myzus persicae* (Sulzer), which is distributed worldwide. In potato crops the transmission of PLRV and PVY depends on the number of infected aphids in the field. The wide spread use of insecticides by growers is expensive and can result in resistance developing in the aphids. Because of this and the negative environmental impact of insecticides the use of host plant resistance is an attractive alternative way of protecting crops.

Table 1: The assessment of resistance to single PVY strains of somatic hybrids (H2; H7) between *S. tarnii* + potato cv. Delikat, BC_1 and BC_2 lines: PVY incidence following mechanical inoculation of 4-5 plants per genotype in a greenhouse using the following strains (of isolates): PVY^N (CH605) – **N**, PVY^O (205) – **O**, PVY^C (Q3) – **C**, PVY^{NTN} (Linda) – **NTN**, $PVY^{NA\text{-}NTN}$ (Nicola) – **NTN*** PVY^NW (Wilga O) – **W, S – susceptible, R – resistant,** ** - plants from a climatic chamber).

Genotype	Number of plants tested/infected						
	N	O	C	NTN	NTN*	W	PVY
cv. Sonate	**5/5**	**5/5**	**5/0**	**5/5**	**5/5**	**5/5**	**S**
cv. Delikat	**5/5**	**5/5**	**5/0**	**5/5**	**5/5**	**5/5**	**S**
S. tarnii**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
H 2	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_1 ∑20 lines	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_2 2/80/2	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_2 2/80/4	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_2 2/80/5	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_2 2/80/8	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_2 2/80/9	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_2 2/80/10	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_2 2/80/1	**5/5**	**5/5**	**5/0**	**5/4**	**5/5**	**5/3**	**S**
BC_2 2/2/80/3	**4/4**	**5/5**	**5/3**	**5/4**	**4/4**	**5/5**	**S**
BC_2 2/80/6	**4/3**	**5/5**	**5/3**	**5/5**	**5/5**	**5/4**	**S**
BC_2 2/80/7	**5/5**	**5/5**	**5/3**	**5/3**	**5/5**	**5/5**	**S**
H 7	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_1 ∑12 lines	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_2 7/53/1	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_2 7/53/2	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_2 7/53/3	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_2 7/53/6	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_2 7/53/8	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_2 7/53/10	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**5/0**	**R**
BC_2 7/53/4	**4/4**	**5/5**	**5/4**	**5/5**	**4/4**	**5/5**	**S**
BC_2 7/53/5	**5/5**	**5/5**	**5/5**	**5/5**	**5/5**	**5/5**	**S**
BC_2 7/53/7	**5/5**	**5/5**	**5/5**	**5/5**	**5/2**	**5/5**	**S**
BC_2 7/53/9	**5/3**	**5/3**	**5/5**	**5/4**	**5/5**	**5/5**	**S**

Aphids are phloem-feeding insects and insert their stylets into plants during the process of selecting a suitable host. One tool for studying aphid feeding behaviour is the electrical penetration graph (EPG) technique (TJALLINGII 1988). EPG monitoring of aphid feeding behaviour was used to search for sources of resistance to *M. persicae* in the wild species *S. tarnii*, somatic hybrids and sexual progeny. One plant of 4 different genotypes was placed in a Faraday cage and the behaviour of aphids on these plants recorded simultaneously for 12 hours. Each plant was used six weeks after transfer from agar (*in vitro*) to soil. This trial of each genotype was repeated 20-25 times with 5 plants. The aphid was attached to the electrode with a wire and connected to a DC amplifier input. The plant electrode was inserted in the soil of the potted plants and connected to the EPG recording apparatus.

The EPG signals analyzed as waveform patterns indicate the stylet position and feeding activity of an aphid (TJALLINGII & HOGEN ESCH 1993). Distinct potential drops are recorded when the stylets puncture the cell membranes, essential for the transmission of non-persistent viruses. Duration of stylet penetration was measured from the recordings. Wave form E represents the phloem phase, which is divided into waveform E1 for sieve element salivation and E2 for phloem sap ingestion.

The waveforms indicating resistance to aphids are the time to the first probe, the number of probes shorter than 3 min (test probes) before phloem phase, the time to first E1 and the time to the first sustained E2 period of longer than 10 minutes indicating 'committed phloem ingestion', the waveform indicating a potential drop is important indication of the potential for acquisition and transmission of PVY (TJALLINGII 1995).

Feeding traces of the aphid species *M. persicae* on different genotypes of *Solanum* spp. reveal that there are differences in the time spent probing these host plants. When probing cultivated potato, cv. Delikat, they needed a short time to reach the phloem and fed for longer on phloem.

The durations of the most relevant waveforms were compared between the cultivar, *S. tarnii,* somatic hybrids, BC_1 and BC_2 lines. The results of this study should clarify if the wild species *S. tarnii* could be characterized as an unsuitable host for potato aphids.

The results of the analysis of electrically recorded feeding behaviour of aphids on plants of the wild species *S. tarnii*, potato cv. Delikat, somatic hybrids and sexual progeny are discussed in the context of possible mechanisms of aphid resistance (ALVAREZ et al. 2006) and their use in the breeding for resistance to diseases and pests in potato.

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TESTS OF TAXONOMIC AND BIOGEOGRAPHIC PREDICTIVITY

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Introduction

Potato breeders commonly use wild *Solanum* species as sources of disease resistance genes. This rich germplasm resource consists of approximately 190 species distributed from the southwestern U.S. to Chile (Hijmans and Spooner, 2001). Collectively, these species represent a more diverse and accessible germplasm resource than in any other crop. They contain genes encoding numerous traits not found in cultivars and represent an especially rich source of disease resistance genes (Hanneman, 1989; Jansky, 2000; Spooner and Bamberg, 1994). Potato gene banks contain large collections of wild *Solanum* species from which breeders can access valuable genetic diversity. However, it is impractical to screen all accessions in a gene bank for resistance to a particular disease. Scientists would benefit from a systematic system to search for resistant genotypes. Taxonomic or geographic information may provide such a search strategy.

Breeders regularly use taxonomic information to choose potential sources of disease resistant germplasm for cultivar improvement. They assume that they can predict the presence of a trait in a taxonomic group for which the trait has been observed in a subset of the group. For example, plant breeders use taxonomy to make their initial choice of related germplasm based on the knowledge that species X is resistant to a particular disease, by choosing other accessions of this or related species. Not all accessions of a species are expected to contain the same traits, but if no prior evaluation data are available, then taxonomy may allow a breeder to make inferences about unevaluated accessions based on knowledge of a subset of the taxonomic group.

Biogeographical variables, such as geography and climate, have also been used to predict the presence or absence of traits in wild plants. Collectors of wild crop germplasm rely on biogeography-based hypotheses of association for guidance. They try to sample from as many ecologically different environments as possible and include extremes of the range of a species. This allows them to collect germplasm that may be uniquely adapted to specific ecological conditions For example, resistance to a certain disease may be present in areas where the pathogen is endemic, but it may be absent in areas that are ecologically similar but lacking the pathogen.

We have designed experiments to empirically test the predictivity of taxonomy and biogeography by associating resistance to white mold (Jansky et al., 2006) and early blight (Jansky et al., 2008) to diverse potato taxonomies and biogeographic data, using a diverse set of accessions of wild relatives of potato *Solanum* section *Petota* and outgroups in *Solanum* section *Etuberosum*. We chose disease tests because wild potato species provide a wide range of resistances to diseases that are easily introgressed into the cultivated potato, and disease resistances in wild species provide one of the major justifications for collecting and storing germplasm in genebanks.

Materials and Methods

For the white mold study, we evaluated 144 accessions of 34 wild relatives of potato, while 144 accessions of 41 wild species were evaluated in the early blight study. Resistance to white mold (caused by *Sclerotinia sclerotiorum*) was evaluated on seedlings inoculated with fungal mycelia. Resistance to early blight (caused by *Alternaria solani*) was evaluated on detached leaves of seedlings that were artificially aged using auxin and inoculated with a conidial suspension of the pathogen. Days until seedling death was measured for the white mold study, while lesion development was evaluated for early blight resistance.

Statistical analyses evaluated variation due to species, taxonomic series (based on an intuitive interpretation of morphological data), clade (based on a cladistic analysis of plastid DNA data), and ploidy. In addition, spatial and environmental variables were evaluated to test whether geographic provenance of accessions could predict disease resistance. These included potential evapotranspiration, actual evapotranspiration, monthly mean low temperature, monthly mean high temperature and monthly rainfall.

Results and Discussion

Significant differences in both white mold and early blight resistance were identified among and within species. The most white mold resistant species were *S. infundibuliforme*, *S. hjertingii*, and *S. megistacrolobum*, while the most early blight resistant species were *S. neorossii*, *S. commersonii*, and *S. tarijense*. Breeders will find resistant accessions in these species, but they could also find susceptible accessions, so these resistances are not species-specific. It was common to find variation among accessions and even within accessions. This is not a surprising finding, since many of the species in this trial are highly heterozygous outcrossers. Even the inbreeding species are expected to be heterozygous because they are maintained by intercrossing at the gene bank. Because of this variability, it is important for breeders to practice a fine screening technique to identify individual clones with desired traits.

The most resistant series in both studies was *Commersoniana*, but it contained only one species, *S. commersonii*. When series containing multiple species were evaluated, none was predominantly composed of resistant or susceptible species. That is, both resistant and susceptible species were found in each series. Similarly plastid-based phylogenetic clades contained both resistant and susceptible species.

In both studies, the tetraploid species were more susceptible than the diploid and hexaploid species, while the diploids were the most resistant ploidy level (early blight) or were no different than the hexaploids (white mold). Again, though, great variation for both resistant and susceptible species were found in each group.

None of the taxonomic grouping criteria consistently identified the most resistant set of species. Therefore, a breeder would not be able to focus on a species, series, clade or ploidy level with the expectation that most or all members in the group will be resistant. Similarly, resistant species could not be grouped by any geographic or climatic variable.

Both pathogens in these studies are air-borne fungi with broad geographic distributions and wide host species ranges. They are likely found throughout the range of wild *Solanum* species. However, local environmental conditions may or may not favor disease development, resulting in variable selection pressure for resistance. These microclimatic influences can not be detected from the biogeographic data used in this study because they are available only on a broad scale. Habitat notes provided by the taxonomists who collected the accessions used in this study may provide the most revealing information about local evolutionary patterns. For example, among the accessions for which habitat data were available, the four most early blight resistant accessions were collected in or adjacent to cornfields. Disease pressure near agricultural sites is likely to be stronger than in more remote wild areas. Consequently, for diseases caused by airborne spores, wild species accessions collected near agricultural fields may be more resistant than those collected in forests or other wild areas. Another microenvironmental variable for which no data are available is pathogen population variability. These studies were carried out under the assumption that resistance to the pathogen isolates derived from cultivated potatoes in Wisconsin confers resistance to isolates found in areas where wild *Solanum* species occur.

We conclude that there is so much variation within the taxonomic groupings of species, series, clade, and ploidy that they can not be used to predict sources of resistance to white mold or early blight. Similarly, geographic distance and climatic parameters are probably too broad to identify particular accessions with resistance to these fungal diseases. This study calls into question the fundamental and widely-accepted assumption that taxonomic and biogeographical variables can predict the distribution of genes useful to plant breeders. Efforts to identify useful wild crop germplasm rely on the expectation that taxonomic relationships can be applied to the selection of useful sources of resistance genes. Although alternative predictors of resistance traits have not yet been identified, it is apparent from this research that other options for the identification of sources of disease resistance genes must be considered.

Since both studies considered resistance to similar pathogens (widely distributed air-borne fungi), it is important to determine whether taxonomy or biogeography are predictive for other traits of interest to potato breeders. We are continuing studies with resistances to other pests and diseases, including resistance to potato virus Y (a virus disease with major resistance genes in wild potato species), the Colorado potato beetle (an insect defoliator with multiple resistance mechanisms in wild potato species), and soft rot (a soil- and tuber-borne bacterial disease). Potato virus Y causes severe stunting and is perpetuated through tuber generations, while the Colorado potato beetle causes severe defoliation and plant death in a short period of time. Soft rot destroys infected tubers and plants. Perhaps taxonomy or biogeography will be more predictive for these traits because selection pressure is stronger than that for early blight and white mold.

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ANTIXENOSIS OR ANTIBIOSIS RESISTANCE IN WILD *SOLANUM* SPECIES AGAINST APHIDS : A CHALLENGE FOR POTATO CROP PROTECTION

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Aphids are important pests for the potato crop, because they vector numerous viruses, such as the potato leafroll virus (PLRV) and the Y potato virus (PVY). At the present time, agricultural practices and climate changes increase risks of aphid population outbreaks. To mitigate or to delay the development of aphid resistance to insecticides, and in response to an increasing demand for environmentally friendly agriproducts, alternative and sustainable methods of crop protection have to be developed. A promising way consists of using the high genetic potential of wild tuber bearing potatoes. In that context, we quantified the resistance of 8 accessions (5 wild *Solanum*) with physiological experiments. Behavioural experiments (i.e. olfactometry and electropenetrography) were carried out to determine the nature of the resistance (antixenosis or antibiosis).

All wild *Solanum* accessions did not repulse aphids. On the contrary, attractive effects of *S. stoloniferum* were observed on *M. euphorbiae*. These results precluded the possibility of plant volatiles involvement in the resistance exhibited by wild *Solanum*. Antixenosis induced aphid behavioural disruption occurring in the different plant tissues, leading to lower phloem ingestion, and in some cases, to the rejection of the plant as a host. In contrast, an antibiosis resistance did not cause either behavioral disruption or reduction of phloem sap ingestion, but physiological disturbances. A generalized antixenosis resistance was found against *M. persicae*. Comparing our results with published works, we conclude that antixenosis is a widespread resistance system in the wild *Solanum* species against *M. persicae*. This is the first time we report antibiosis and antixenosis resistance of wild *Solanum* species against *M. euphorbiae*. Localization of antixenosis factors within plant tissues was very variable between wild *Solanum* species and even between accessions of the same species as *S. stoloniferum*. Variability in resistance was also observed according to the two aphid species. These results suggest involvement of several resistance mechanisms even within a same *Solanum* species. To explain antixenosis, we may hypothesize involvement of physical or chemical barriers at the cuticular or epidermal level, deterrents into the mesophyll or into the phloem and obstruction by phloem proteins or callose into phloem elements. A gene-for-gene or hypersensitive response may be involved as well into all kind of tissues. Antibiosis resistance may be explained by toxic compounds, low nutritional quality, or induced plant response.

Introduction of wild resistant traits in the cultivated potato may allow creation of new resistant cultivars. Moreover, identification of active molecules could lead to biopesticide development.

GENETIC ANALYSIS OF SENSORY AND VOLATILE TRAITS IN POTATO

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Abstract

Hybrid populations between *S. tuberosum* and *S. phureja* have been used to perform genetical studies of organoleptic quality of potato. We have carried out extensive sensory and volatile profiling of a diploid population. This population has also been used to generate a genetic map of ~250 markers. The population shows significant phenotypic variation for most sensory traits analysed, and it has been possible, for the first time, to identify QTLs for sensory characters. Interestingly QTLs for some volatile components of cooked potatoes co-segregate with flavour QTLs. This analysis paves the way for candidate gene studies.

Breeding

PROGRESS IN POTATO BREEDING AT SCRI

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The potato breeding programme at SCRI dates back to the foundation of the Scottish Plant Breeding Station (SPBS) in Edinburgh in 1920. Since then 72 varieties have been bred and released. The presentation will examine the time taken to breed new varieties, the size of the programme and the number of cycles of crossing and selection since 1920, a period of 88 years. The use in the programme of the wild and cultivated species of Latin America will be covered, including the time taken for introgression and base broadening. There will be a discussion of the change from government to commercial funding of potato breeding following the establishment of Plant Breeders' Rights in the 1964 Seeds Act which aimed to encourage commercial breeding. Successes and failures in trait improvement will also be discussed. Finally, future prospects will be considered in terms of end user needs during a period of climate change.

POTATO BREEDING IN BELARUS: OBJECTIVES AND DISTINCT CHARACTERS

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Potato breeding program in Belarus started in early 20^{th} of the 20^{th} century. For this long period more than 100 varieties have been released. Taking into account climatic, social and cultural characteristics of Belarus, it is necessary to point out main items, influencing the choice of potato breeding objectives:

a) potato has been one of the main food crops for Belarusians for the last two centuries;

b) 90% of potatoes are grown on small house plots or gardens where disease and pest control is limited;

c) changes in consumer preferences concerning maturity class, tuber appearance and cooking types.

All above mentioned give us the possibility to speak of potato breeding in Belarus as breeding varieties which are highly yielding and having high table and processing qualities. On the other hand the chance of breeding "perfect variety" is too low and we have to tackle the most significant problems for local growers, such as blight resistance and high adaptable potential for different soil types and weather conditions. It means that general requirements to potato varieties are:

- high yield;
- long tuber dormancy;
- early tuberisation and intensive tuber growth;
- resistance to diseases and pests;
- resistance to mechanical damage;
- stability of main properties.

In breeding for earliness main attention is put on early marketable yield than physiological earliness (Alsmick, 1933). The use of original model for parental choice and method of selection of the best lines at the early generations of a potato breeding program gave us the possibility to breed early varieties with high yield and good table qualities (Makhanko, 1999, 2000) (Figure 1).

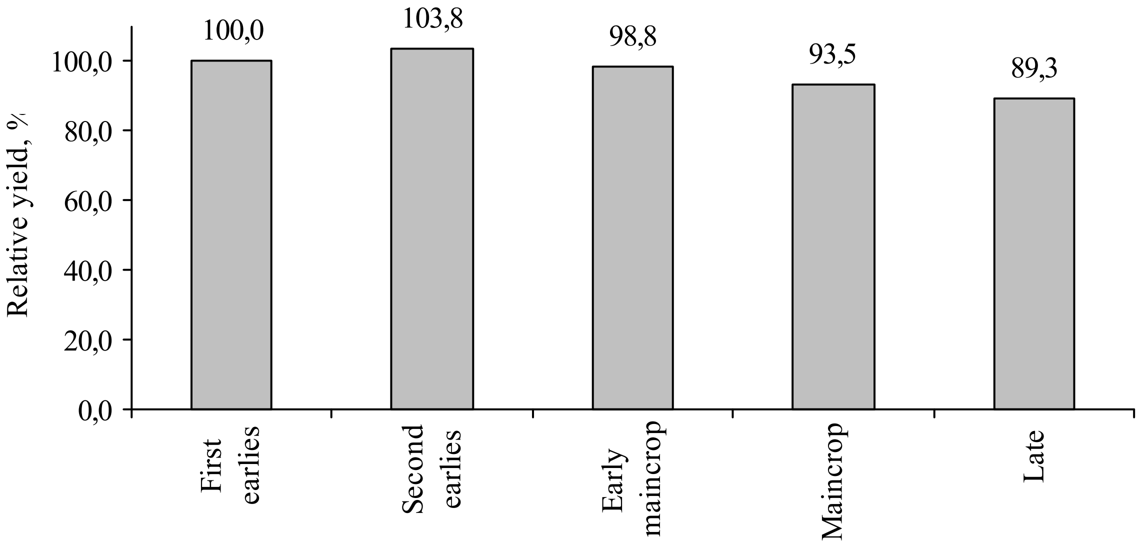

Figure 1. Relative yield of potato varieties of different maturity classes in official trials (1996-2006).

Potato breeding for processing after cold storage is one of the most promising objectives in modern potato breeding. The main steps of this work are as follows:

- parental choice on chipping color after harvest and 3 and 5 months of cold storage (2-3°C) without heating;

- progeny assessment from different types of crosses and selection of promising populations;
- assessment of selected clones for agronomic traits.

Potato breeding for high starch content is traditional objective for Belarusian potato breeding program (Alsmick, 1979). It is evident that more than one cycle of crossing is required for success in this work. The next special features from our point of view are strongly recommended in breeding for high starch content:

- using of donors of high starch as male parent;
- stepped crosses.

At the latest stages of potato breeding program in Belarus advanced breeding lines are put in trials for different agronomic and quality traits at eight sites differing on climatic and soil conditions. 19 advanced selections and 5 standard varieties have been assessed for dry matter content (DM) and reducing sugars content (RS) for two years. Differences, both between and within maturity classes, were recorded on both assessed parameters ($P<0.01$). DM was found to be quit stable character depending mainly of genotype (58.76%), effect of growing site and year was minimal (11.1 and 7.84% respectively). Influence of environment on RS was predominant (36.97%).

Analyses carried out at three harvest dates from mid July showed that in first early and second early varieties threefold decrease of RS was recorded. Those of maincrop varieties decreased more considerably (4-8-fold decrease).

Research strategy in potato breeding directs at wide use of germplasm from primitive, cultivated and wild species via tetraploid interspecific parental lines, dihaploids and somatic hybrids for breeding varieties for different purposes (Figure 2). Also big efforts are put at parental choice and selection of the best lines at the early generations of a potato breeding program.

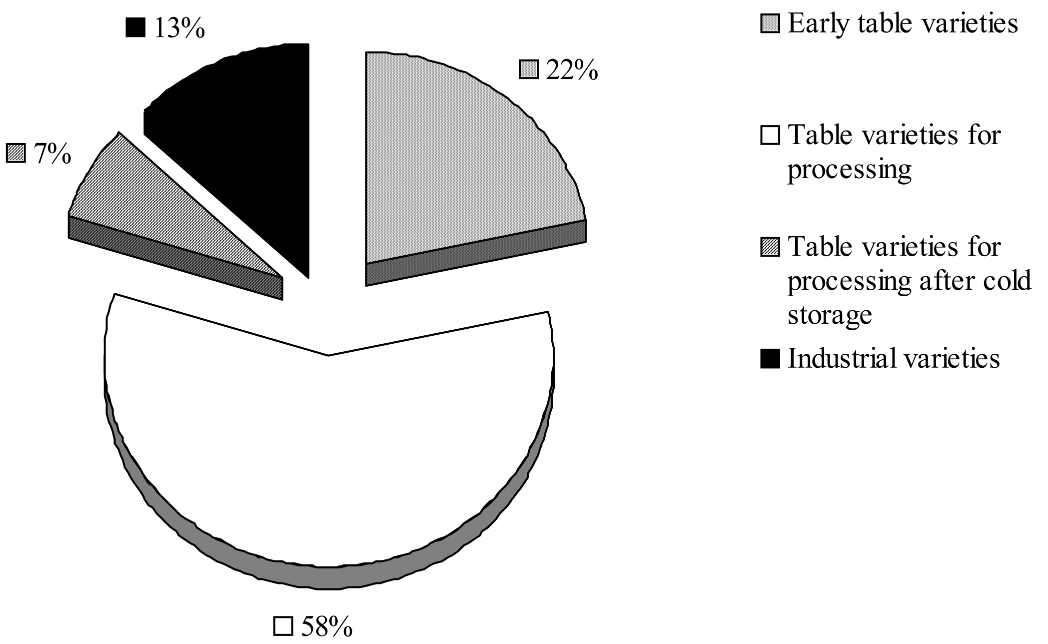

Figure 2. Outlook for potato breeding in Belarus.

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RESISTANCE BREEDING AGAINST *PHYTOPHTHORA INFESTANS* – A COMPLEX APPROACH

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Low progress in resistance breeding against *Phytophthora infestans*, which has been actively conducted over last 50 years in potato breeding, might be explained by limited knowledge on pathogen, host and their interaction. To enhanced late blight resistance of new cultivars our studies are directed on both resistance of host plants and the characterization of *P. infestans*.

New sources of resistance to *P. infestans* have been identified among *Solanum* species, like *S. phureja*, *S. verrucosum*, *S. microdontum* , *S. michoacanum, S. ruiz-ceballosii* and *S. kurtzianum*. ((Jakuczun and Wasilewicz-Flis, 2004). Parallel to the introduction of found resistances into cultivated genepool via recombination breeding, the research is focused on determination of their inheritance by the progeny and on mapping these sources in the potato genome. There are determined genes and QTLs responsible for leaf and tuber resistance to *P.infestans* and QTLs responsible for the length of vegetation period (Śliwka et al. 2007). We introduce molecular markers for selection of late blight resistant forms in the breeding program. The PCR marker for the gene *Rpi-phu1* (mapped on chromosome 9) is used for selection of resistant breeding lines both on diploid and tetraploid level (Śliwka et al., 2006a). In recent years somatic hybrids were obtained for resistance sources non crossable to *S. tuberosum.* Resistance of obtained somatic hybrids *S. tuberosum* (+) *S. nigrum* - has been determined (Zimnoch-Guzowska et al., 2003).The group of *S. nigrum* accessions has been characterized and study on genetic determination of inheritance of *S. nigrum* resistance has been undertaken (Lebecka, 2008).

For actually grown cultivars we are intended to identify cultivars most stable in resistance expression. Thus the set of popular Polish resistant cultivars has been tested in various locations of the country for several seasons (Tatarowska et al. 2003).

Large collection of *Phytophthora infestans* isolates has been studied, which is representative for Polish population of the pathogen. Isolates are characterized phenotypically and genotypically. We monitor the pathogen population each year (Śliwka et al. 2006b). For selection of *P.infestans* resistant forms in breeding program, both field screening under strong infection pressure and laboratory assays are applied. For laboratory assay of leaflet and tuber resistance most pathogenic isolates are selected out of 10-15 most complex ones. Isolates are tested for virulence against Black's differentials and set of identified sources of resistance, what is in parallel the characterization of studied resistance sources.

We assume, that well developed procedures of screening for resistance, monitoring of the pathogen, wide utilization of new sources of resistance, use of molecular markers in selection and in addition soon perspective of utilization of *cisgenesis* in modification of susceptible cultivars, lead to significant improvement of late blight resistance in grown potato cultivars.

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Varietal assessment

THE VALORISATION OF THE ITALIAN TYPICAL POTATOES: USE OF THE CHEMICAL INDEXES FOR THEIR CHARACTERIZATION

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Introduction

The statement of the European Commision clearly indicates the importance of multifunctionality for European agricultural policy. In spite of this the evaluation of the crop productions is now oriented to give additional values to the geographical origin and to the environmental and socio cultural aspects of the agricultural goods. In this context, arose a very high interest for the typical products, a sector where Italy is well represented at European level in spite of one of the greater percentage of typical products. Consumers appreciate the peculiar distinctive sensory characteristics of these foods (Lovatti and Castagnoli, 1999) and consider them safer than the others because of the high number of controls carried out on them. Moreover, a big public interest is also attributed to typical food products for their role in stimulating the economy of some protected areas, in preserving the biodiversity linked to local crop varieties and animal races and moreover in keeping alive ancient traditions in food processing. For all these reasons, together the higher cost of production, the selling prices of typical and traditional products are higher compared with the commercial products. Therefore, there is a considerable interest in new methods that permit geographical and variety identification of alimentary products, and that consent the recognition of possible frauds.

Different are the system way for these studies: the identification of genetic resources, using genomics and proteomics tools (Moisan-Thiery *et al.*, 2005). They are quite useful and easy to use. Regarding the identification of geographical origin, different screening methods have been adopted based on the analysis of some organic compounds content, such as phenols, amino-acids, biogenic amines, aromatic alcohols and other volatile substances (Brescia *et al.* 2004; Duckham *et al.* 2002; Forgács and Cserháti, 2003; Montouto-Gran *et al*, 2002; Oruna-Concha *et al*, 2002b; Tedone *et al*, 2007), lipid acidic composition and isotopic ratio between elements present in the organic matrix (Del Signore *et al*, 2004. Guerzoni, 2004, Padín *et al.*, 2001; Peña *et al.*, 2001). An additional difficulties is the modification of the foods products during its shelf life. With regard to the above mentioned information, a biennial research program founded by the Italian Ministry of University and Research (MIUR) entitled *Environment, genotype and typicity in potato and artichoke* was implemented, involving three regions: Apulia, Sicily and Tuscany. In this paper we resume the activity carried out on potato, whose aim was to define useful tools to link a typical food product to a geographic production area.

Materials e methods

The research was carried out during the years 2006/2007. Three typical varieties of three Italian regions, where the potato cultivation is typica: a) Tuscany, in the Pratomagno location, on the mountain (Cetica), where a local potato variety, named ‘Rossa di Cetica’, is cultivated; b) Apulia, in the Salento place (Ugento), in an area where it is traditionally cultivated, in extra-seasonl cycles, an ancient German variety, Sieglinde; c) Sicily (Cassibile), in extra-seasonal cycles also, where usual is the cultivation of the variety Arinda. The three varieties above mentioned were grown in each one experimental sites. Tuber seeds, collected in each area of provenience, were utilised for each sowing. In Apulia and Sicily, the early crop cycle was performed, with sowing in January and harvesting in late May-June, while in Tuscany the summer crop cycle was carried out, with sowing in May and harvest in September.

Plant material (tubers) analysis: Tuber samples, at harvest time, were calibrated and the 40-60 mm interval size was used for the following analytical determinations:

- Macro- and microelement determination, the determinations were effectuated by University of Florence on pulp and skin of the rinsed tubers. The determinations were performed on the soil used for the experimental schemes of the three locations. The analysis, developed with an Inductively Coupled Plasma-Optical Emission Spectrometry (ICP), allowed to determine the amount of the following mineral elements: Al, Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Sr, and Zn.
- Aromatic compounds determination, the procedure were developed by the DSPV, University of Bari in collaboration with the Center of Research Bonomo of Andria (Bari) and performed on cooked and peeled potatoes through an Headspace Solid Phase Microextraction (SPME) analysis using a Supelco fiber followed by a GC/MS analysis of the adsorbed compounds.
- Phenolic acids: this determination was performed on the tuber pulp through a HPLC

Results

- Aroma: were identified 40 volatile organic compounds (VOCs) of the following categories: hydrocarbons, aldehydes, furans, terpenes, carboxylic acids, esters, alcohols, phenols, other classes (Fig. 1). Some compounds were representative of the genetic variability because of the presence in higher quantity in some varieties, while others were linked to the geographical origin. Regarding the geographical origin of the tubers: 2-Ethylhexyl 2-ethylhexanoate and Heptane-2,6-dimethyl were absent in potatoes cultivated in Tuscany, and very scarce in those obtained from Sicily. Heptanal, Octanal and Nonanal were present in higher concentrations in Sicily, whereas Butanal 2-methyl, Butanal 3-methyl and Tridecane were more abundant in potatoes from Tuscany.

- Phenolic acids: The data collected were indicative of the varieties: the phenolic acids content was higher in the pulp tissue of Rossa di Cetica, followed by Sieglinde and finally Arinda (Fig. 2).

- Macro and microelements in the tubers: the elements detected were present in measurable amounts in both the skin and pulp. The analysis of variance showed that in the pulp tissue, there were significant differences for locality in all the elements, with the exception of Al, Cd and Co. Most elements (B, Ba, Ca, Cd, Cu, Fe, Mg, Mn, P, Sr, V, Zn) were also significantly different for variety. The PCA (Principal Component Analysis), conducted on the variables with the greatest differences between locality (Ba, Ca, Cu, Fe, K, Na, Ni, P, Sr, Zn) allow a net separation into three groups according to locality (Fig. 3). It was not, however, possible to separate samples according to cultivar. Nonetheless, when performing the PCA within locality for the 7 variables (B, Cd, Cu, Fe, Mn, V, Zn) showing the greatest differences for variety, it was possible to completely separate the samples derived from Cassibile (Sicily) into groups. In the samples derived from Ugento (Apulia), an overlap was evident between Rossa di Cetica and Arinda, whereas a minor degree of overlap was shown between Arinda and Sieglinde in Cetica (Tuscany).

The results showed a positive correlation between the soil pH and Ca and Sr in the tubers (Fig. 5). In contrast, a negative correlation was evident between soil pH and Ba and Ni. In few cases (K, Na, Sr) there were positive correlations between the concentration of the elements in the soil and in the tubers. A negative correlation was found between the exchangeable soil Ca and the microelements Ba, Fe and Ni in the tubers.

Conclusions

The study of volatile aromatic compounds identified many differences according to geographical origin and variety. Therefore, this methodology is important in verifying the typicality of the products. It is also useful in identifying the chemical components responsible for the different aromas on a sensory level. The phenolic acid and alkaloid composition, even though variable within each trial, may also contribute in the characterisation of plant samples. The concentration of phenolic acids is mostly linked to genotype, whilst the presence of the alkaloids appears to be mostly dependent on the locality.

Mineral element analysis coupled with subsequent multivariate statistical analysis proved to be extremely useful in the characterization of plant material. The results obtained on the mineral element determinations, in accordance with previous research, permitted the correct classification of 100% of the samples according to the geographical origin. With respect to the genotype, although it was not possible to completely separate the varieties in two of the three locations, differences in mineral element content together with suitable genetic analytical techniques, were sufficient to permit variety identification.

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Figure 1 – Volatile compounds generated during cooking. Amounts of individual components, expressed in semi-quantitative terms, as GC peak area relative to the GC peak area of the internal standard (2-pentanone), injected at the concentration of 1 mg/kg of tubers.

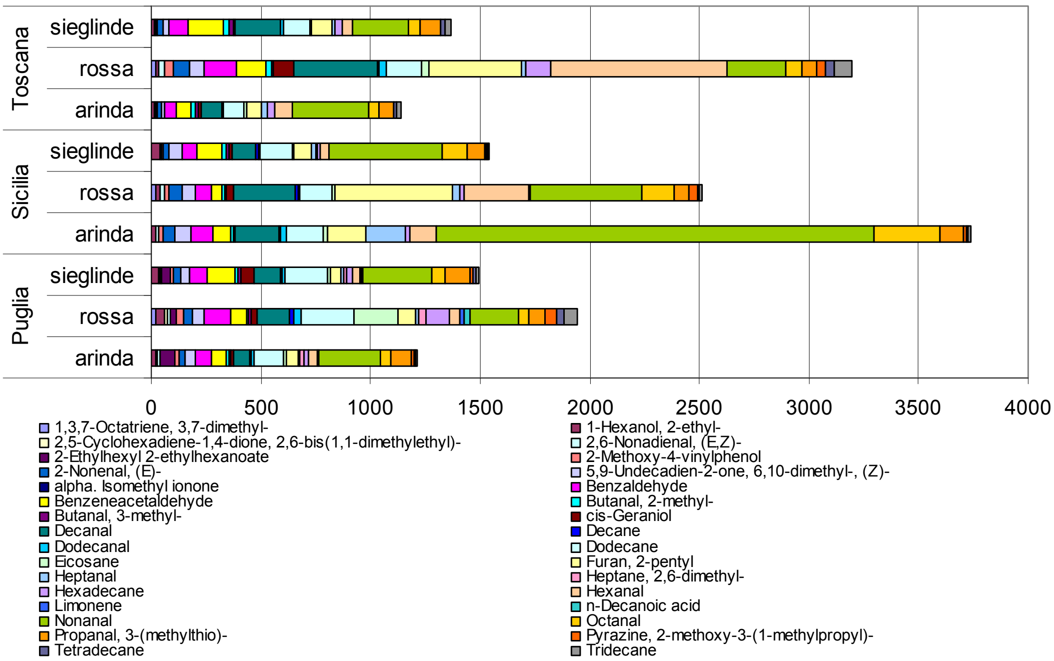


Figure 2 - Concentration of phenolic acids (mg/g of freeze-dried pulp) in potato tubers cultivated during 2006. Each value is the mean of 4 replicates.

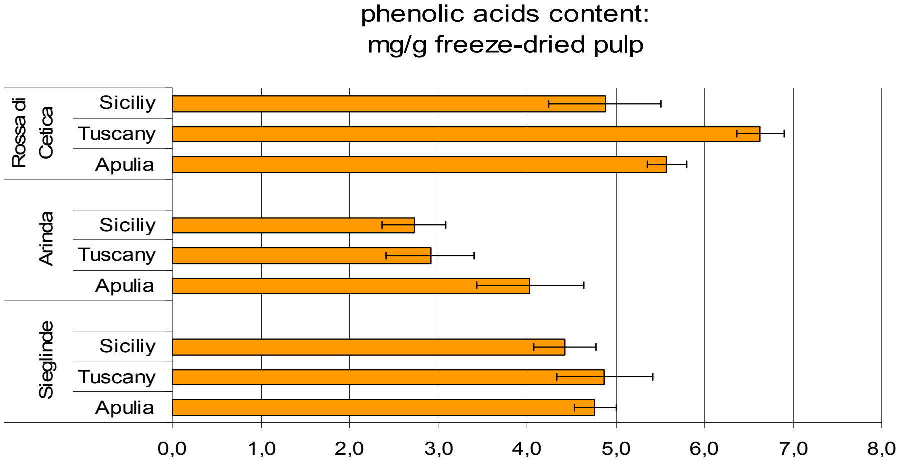


Figure 3– Graph of Principal Component Analysis conducted on the 10 elements of potato pulp with the greatest differences between locality. Legends: first 2 letters refer to experimental location, (BA Apulia, CT Sicily, FI Tuscany), the last letter refers to variety (A = Arinda; R = Rossa di Cetica; S = Sieglinde).

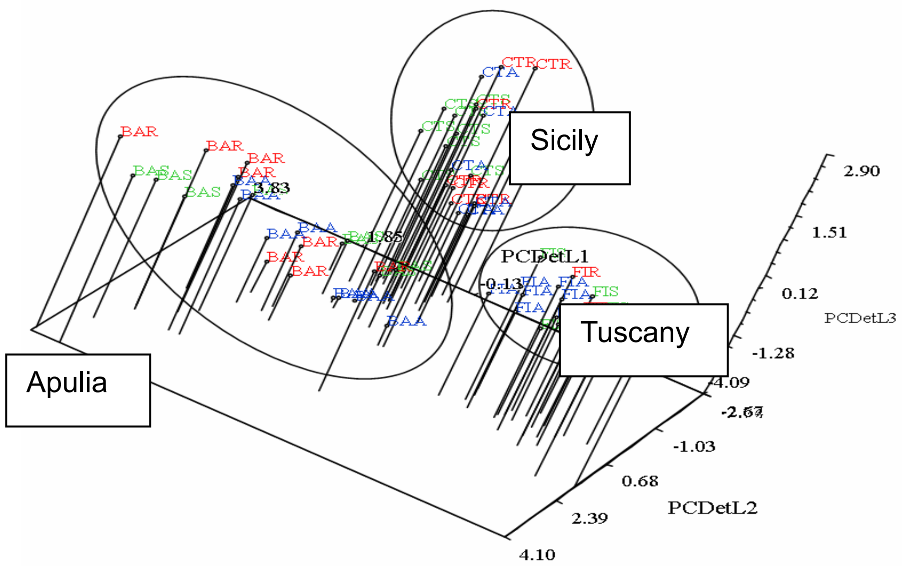
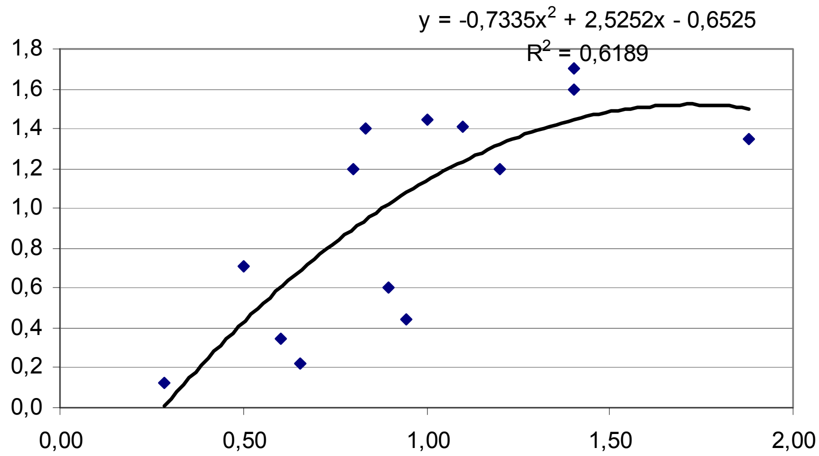


Figure 4 – Correlation between K, Na, Sr content in the soil and in the tubers (index value of the elements respect to the average)



VARIATION OF GROWTH AND DISEASE CHARACTERS BETWEEN CLONES OF POTATO (*SOLANUM TUBEROSUM* L.)

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Introduction

In a potato nuclear stock collection each cultivar is represented by only one or a few clones. Ahloowahlia (1994, 2000) showed that micropropagation by stem cutting is genetically very stable. Clones are normally assumed to be genetically identical. A clone is defined here as a plant established from a main shoot or an axillary bud from a mother plant true to type and all the material descending from it. Few results from field experiments examining the homogeneity of clones of the same cultivar at different locations using disease-free seed have been published. Most results indicate a rather high genetic stability of clones except for few variable varieties (Wright 1983, Gustafsson 1984). On the other hand, Rosenberg & Kotkas (1990, 1993) and Kotkas & Rosenberg (1999) found substantial differences between potato clones for important characters. In order to investigate further the stability of clones of micropropagated potato, a range of cultivars bred in Sweden, Denmark and Estonia were grown at three locations over three years to examine whether any clone was distinct from other clones of the same cultivar and whether such a difference was consistent over locations and years. Only a selection of results from the comprehensive trials is presented. The results are *in print* in Potato Research (Nielsen *et al*. 2008).

Materials and methods

The investigation included 10 clones of each of the cultivars Vigri and Ants from Estonia, Matilda from Sweden, and Folva from Denmark. The potato cultivars existed in advance as meristem in vitro cultures and were tested for freedom from pathogens stated in the EU Commission Directive 92/103/EC. Uniform seed tubers for the first two years of field trials were provided from Finland and for the last year seed was harvested from one of the field trial the previous year. The trials were laid out as randomised complete block designs with four replicates, and the clones were randomised within the blocks. The plots consisted of two parallel rows of 20 plants each. Trials with Folva were carried out in Denmark, Sweden and Estonia, while the three other cultivars were grown only in Sweden and Estonia.

Several growth and disease characters were recorded. Among these were emergence (a 1-9 scale, 1 = 0% and 9 = 100% emergence), number of tubers (all tubers in one row), number of stems (counting the primary stems on the 10 central plants in each row), time of flowering (a 1-9 scale, 1 = all flowers in the bud stage and 9 = end of flowering), dry matter % (measuring the specific gravity), yield (weight in kg of the tubers of all plants per plot) and size grading (a size index was constructed).

Statistical methods. Because the size grading was done differently in each country it was not possible to compare the grading over countries. Therefore a size index was constructed. This index was calculates as a weighted average of the minimum and maximum size in each grading, using the actual weight of tubers falling in each grade.

For each combination of cultivar, country and year all characters were analysed using the standard linear model for the analysis of a randomised complete block experiment. This model was first used to check the assumptions for such an analysis. Residuals were calculated and visually assessed in order to check the homogeneity of variances and normality and to spot possible outliers. This showed that some characteristics needed to be transformed in order to stabilise the variances. For the characteristics based on counts (e.g. number of plants, number of stems), a square-root transformation was used. A logarithmic transformation was applied in most other cases (e.g. weight of tubers). Outliers that were thought to be associated with for example, machinery damage, missing plants or the misreporting of data were excluded from the analyses.

In order to examine whether the differences found were consistent over locations and year, the data for each cultivar was analysed as series of trials using two different models. In both of these models plot level data were used and the effect of clone was included as a fixed effect. In the first of these two models data from each cultivar and year were analysed separately including the effect of location, replicate within location, location by clone and plots as independent normally distributed random effects. In the second all data from each cultivar were analysed together (over years and locations) including the random effect of location, year and location by year as well as the interactions between these three effects and clone. In both these models the random effects of plot were assumed to have a location specific variance and the individual clones were compared against the mean of the all other clones using a t-test. (More details on the statistical methods are given in Nielsen *et al.* (2008)).

Results

During the first year of seed propagation, two clones showing clearly visible deviating morphological traits were eliminated, meaning that the investigation only included clones of each cultivar that could not visually be separated in the field.

Each character was analysed according to the model described earlier. The residual standard deviations were tabulated. The number of times the differences between clones were significant at the 0.1%, 1% level, and the 5% level of significance were recorded. Out of the 412 tests that could be performed, 46, 81, and 124 were found to be significant at the 0.1%, 1%, and 5% level, respectively. This is far more than could be expected by random chance if there were no true differences between the clones. When summarised for each country, year, cultivar or character, it was found that more significant results than expected were found in all countries, all years, all varieties, and most characters. The characters: emergence, number of tubers and stems, time of flowering, dry matter, yield and size index were examined further in order to check the consistency of the significant differences. The analyses showed that some differences were consistent over more than one trial. A closer analysis of the significant variations between clones of the characters revealed that most deviating data could be referred to a few clones. An example of such a deviating clone of Matilda is shown in Table 1.

Table 1. . Three characters, where clone 3 of Matilda deviates significantly from the mean.

Character:	**Number of tubers**			**Yield**			**Size index**		
Trial	Cl. 3	Mean	Sig	Cl. 3	Mean	Sig	Cl. 3	Mean	Sig
Estonia 1999	12.0	11.1	*	24.0	24.0	n.s.	41.9	42.9	n.s.
Estonia 2000	18.0	16.1	**	29.1	29.8	n.s.	43.1	46.2	**
Estonia 2001	12.4	11.3	n.s.	18.5	18.8	n.s.	36.7	38.9	**
Sweden 1999	18.6	16.5	***	37.9	37.3	n.s.	43.5	45.3	***
Sweden 2000	9.2	8.2	**	17.4	18.9	**	42.5	45.2	***
Sweden 2001	9.2	8.9	n.s.	30.2	29.7	n.s.	51.4	51.5	n.s.

Notes. Sig: significance. n.s.: non significant. *: significant at the 5% level. **: significant at the 1% level. ***: significant at the 0.1% level. Cl. 3: clone 3.

Table 1 shows that clone 3 of Matilda produced significantly more and smaller tubers in 4 of the 6 trials and although the yield of the cultivar varied over years and between localities it only differed significantly from the mean in one trial out of 6.

Discussion

The statistical analyses showed that far more significant differences between clones were found than could be expected by random chance if there were no true differences between the clones. Most of the significant differences were related to a few clones.

Some characters are expected to influence other characters. Late emergence causes a lowering of the yield under normal growing conditions and there is a positive correlation between the number of stems, the number of tubers formed and tuber yield, but the number of stems has a greater influence on the number of tubers than on the yield. This was seen with Matilda, clone 3, where the high number of tubers also seemed to influence the tuber size index in the low direction. These results were consistent over years and in different countries. A clone of Folva showed an opposite trend with a combination of a late emergence, a low number of stems, a low number of tubers and a low yield.

Even though some of the deviations were statistically significant, it is necessary also to consider whether the real differences are sufficient to pay for an extended test of clones, to be able to select the best ones. "The best or the worst one" is not an unambiguous concept, however, as it depends on the interest behind it. Important economic characters are yield, starch content (dry matter), and number of tubers for seed tuber production. For one clone of Folva the average reduced yield over eight trials reached 10% (3.6 t/ha) of the average yield and 9.5% fewer tubers (45,000 tubers/ha). One clone of Matilda resulted in 1% lower yield (0.2 t/ha) than the average and 10% (49,000 tubers/ha) more tubers than the mean over 6 trials. A clone of Vigri gave 2% (0.4 t/ha) less starch than the mean over 6 trials. Only the clone of Folva reached a reduced yield that can be described as economically important and even though it may not be possible to justify the high costs of at least two years comprehensive field trials of new clones to enable selection of a clone from further propagation.

The four cultivars differed in how many of their clones' characters that significantly deviated from the mean. No differences were seen in Ants, whereas some clones of Folva, Matilda and Vigri differed significantly from the cultivar mean for some characters. The differences found were more frequent than those reported by Wright (1983) and Gustafsson (1984). The results support Rosenberg and Kotkas (1990, 1993) and Kotkas and Rosenberg (1999), although the size of the differences was not as great.

The demonstration of existence of significant clone differences shows that in potato nuclear stock collections it is risky to have a cultivar represented by a single clone only. The more clones that are kept to provide the background for the propagation of a cultivar, the less is the risk that the variety differs from the original.

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DROUGHT TOLERANCE OF SELECTED POTATO CULTIVARS

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SUMMARY

The aim of this study was to compare drought resistance of three potato cultivars: Satina, Tajfun and Violet Fleischiege. Yield decrease, relative water content (RWC), content of chemical compounds and their distributions in potato tubers was estimated in cultivars harvested under drought conditions and with optimal water supply. Dry matter content increased in tubers of all tasted cultivars due to stress factor. Starch content under drought condition deceased at all tubers of tested cultivars except from Satina tubers. Water deficit caused increase of nitrate (V) in all cultivars at stolon part of tubers as well as at top part of tubers. Drought condition made the acceptable for potato nitrate content of 200 mg/kg of fresh matter was overdrawn. At all varieties total sugar content increased except from Satina. Under drought condition heterogeneous distribution of chemical compounds in potato tuber were observed.

INTRODUCTION

It is well known that potato comparing to other crops is very sensitive to drought conditions and even very short period of water shortage has negative effect on consumption and technological criteria of potato tubers. During drought stress tuber yield may be considerably reduced even up to 50% (Głuska, 2004; Boguszewska, Głuska, Nowacki, 2006). Variations in water supply of potato plants may also cause irregular distribution of carbohydrates in tubers. It influences on production efficiency, colour of fried products, texture and taste of boiled potato. Increasing amount of nitrate, antinutritional substances, is also the effect of drought condition. Different potato cultivars have different reaction to drought.

The aim of this study was to estimate yield decrease, relative water content in leafs (Barr, Watherley 1962) and content of chemical compounds and their distributions in potato tubers.

MATERIALS AND METHODS

Pot experiment was carried out for 3 potato cultivars: Satina, Tajfun and Violet Fleischiege in years 2006 - 2007. Plants were planted into 12 l pots. The pot is cylindrical pipe (di = 28, height 30 cm). Filled with peat – sand substrate, pH -5,5. Two combination of water supply were used. First were plants watered with optimal amount of water, second plants which were under drought conditions (water supply was completely stopped for two weeks in three weeks after tuberisation period).

Starch content was determined using Ewars method, reducing sugar using spectrophotometry method with dinitrophenyl reagent, sucrose using spectrophotometry method with anthrone reagent. Nitrate V content was determined using ionoselective nitrate electrode by multi-purpose equipment CX-721 Elmetron.

RESULTS AND DISSCUTION

Discontinue of water providing made high drought stress for pot plants, which is not possible on field condition. Yield range of control plants was from 554 to 995 g per plant and plants under drought stress were appropriate 308 to 547 g per plant. High differentiation between combinations was observed. Also the impact of cultivar and drought on yield level was significant (tab 1). It indicates on very high differentiation of potato cultivars. The effect of drought on potato cultivars is the aim of many authors [2,3]. Their results confirm difference between cultivars.

Yield structure also changed under drought condition. Yield structure of watered plant contain mainly tubers 40 – 60mm. Main share of yield in plant under drought stress were tubers under 30 mm and 30 - 40 mm.

Table 1. Drought influence on yield (g/plant).

Cultivar / Combination	2006			2007		
	K	S	mean for cultivars	K	S	mean for cultivars
1. Satina	805	444	625	995	503	749
2. Tajfun	716	406	561	812	547	679
3. Violet Fleischiege	554	308	431	630	445	537
Average for combination	692	386	539	812	666	739

* K- control plants; S – plants into drought stress;

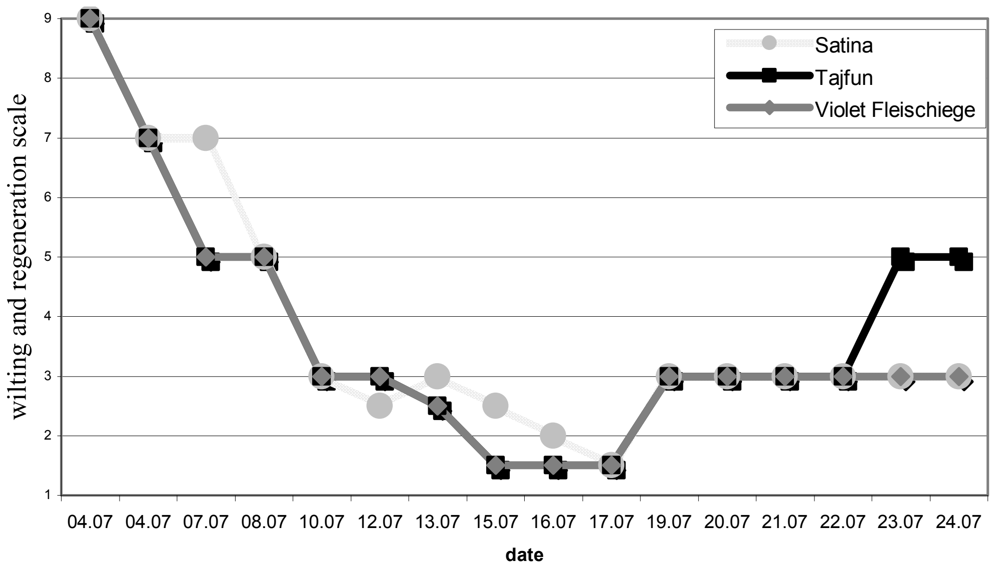
Table 2. Relative water content of potato leaves after drought period.

Cultivar	K	S	RWC (%) (K- S)/K*
Satina	87	48	45
Tajfun	85	52	39
Violet Fleischiege	85	52	39

* K- control plants; S – plants into drought stress;

An average value of relative water content for plants with normal water supply was in a range 85 -87 %, and RWC measured after 2-week of drought was from 48 to 52%. The highest difference of relative water content was estimated at Satina cultivar. Taking into account wilting and regeneration scale with the help of with it was possible to observe aboveground parts of plants in drought stress conditions. The best recovery process was observed at Tajfun cultivar (fig.1).

*Fig. 1. Drought influence on potato plant wilting**

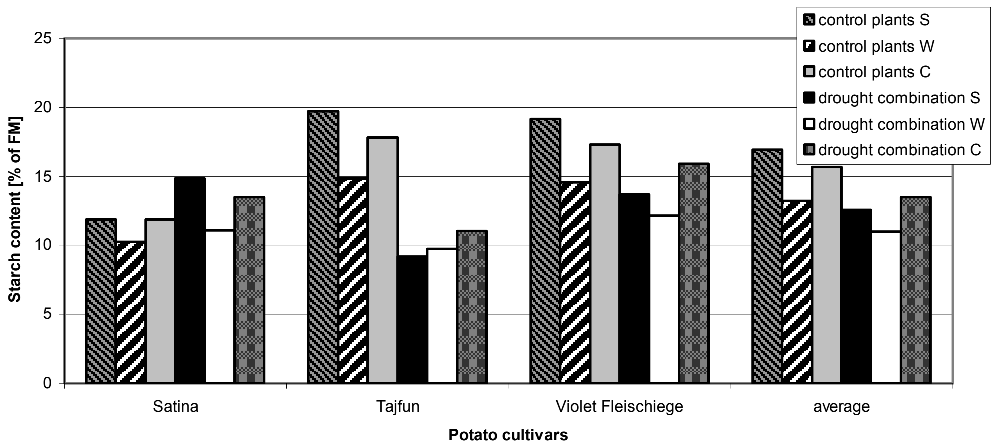


*wilting and regeneration scale 1-9 where 9 is without wilting, 1- total wilting

Starch content due to drought stress decreased at stolon part of tubers as well as at stem end part of tubers at all tested potato cultivars except from Satina (fig. 2). The highest changes of this compound were observed at stolon parts of tubers. Heterogeneous distribution of starch influences on texture and consistency of potato flesh causing e.g. heterogeneous cooking of potato.

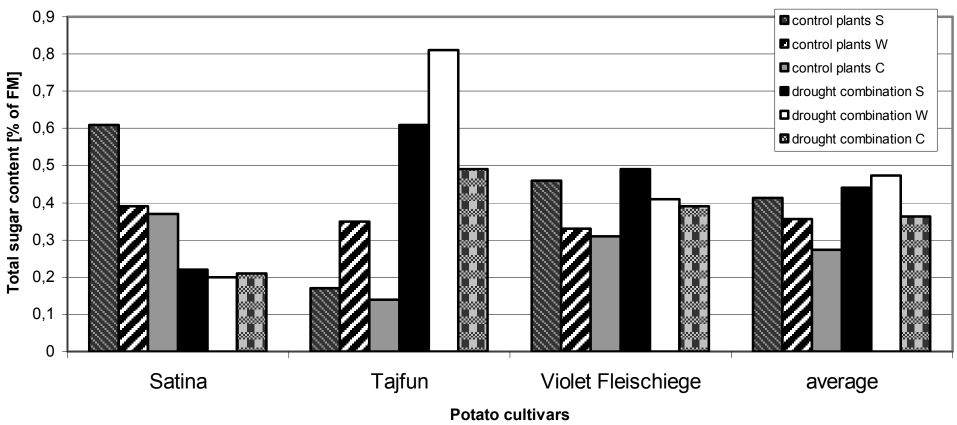
Due to the stress factor it was observed changes of total sugar content at potato tubers. The amount of total sugar content should not be higher than 0.5 % at potato tubers for fried and sterilised products. The levels above 1 % of this compound in fresh mass changes taste of potato making tubers sweet. At Tajfun and Violet Fleischiege tubers the level of total sugar content increased. The highest increased, above 0.8 %, was estimated at stem and part of tubers in Tajfun tubers (fig. 2).

Fig. 2. Drought influence on starch content and its distribution at potato tuber*



****S- stolon part of the tuber, W – top part of the tuber, C – whole tuber***

Fig. 3. Drought influence on total sugar content and its distribution at potato tuber*



*S- stolon part of the tuber, W – top part of the tuber, C – whole tuber

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IN VITRO SCREENING OF POTATO AGAINST WATER-STRESS MEDIATED THROUGH POLYETHYLENE GLYCOL IN SINGLE- AND DOUBLE-LAYER MEDIA

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Improvement in root traits is considered to be important for developing drought tolerant genotypes (Iwama and Yamaguchi 2006). However, measurement of root traits in field trials is cumbersome and time consuming, thus limiting the number of genotypes that can be screened. Further, due to genotype x environment interaction and a large error in recording such traits under field conditions, a genotype is required to be evaluated over years and locations. After several years of field evaluation of a segregating population of Konafubuki x Iris Cobbler for root characteristics, the Crop Science Laboratory of Hokkaido University, Japan identified a potato genotype 'IWA-1', which is significantly superior to other genotypes in root dry weight. Gopal and Iwama (2007) and Gopal et al. (2008) evaluated this genotype along with two other genotypes, (which were at par for root dry weight) under in vitro conditions for root growth. Gopal and Iwama (2007) reported that by induction of limited water-stress by addition of sorbitol or polyethylene glycol in MS medium it was possible to differentiate the potato genotypes for their roots growth in microtuber derived plantlets. Gopal et al. (2008) found that by increasing the amount of agar in the MS medium the plantlets derived from nodal cuttings could also be used for differentiating the potato genotypes for their root growth. Importantly, the in vitro root growth in different potato genotypes in both these studies reflected the observed relative root dry weight of these genotypes under field conditions in plants grown from normal tubers. Under field conditions, tillage makes the plow layer of soil softer than the underneath one. The drought tolerant genotypes are known to have deeper roots with higher capacity to grow into hard pan, and it was so in 'IWA-1", thereby resulting in its higher root dry weight than other genotypes (Iwama and Yamaguchi 2006). In the present study, shoot and root growth in three potato genotypes (IWA-1, IWA-3 and IWA-5) was studied in single- and double-layer media with variable levels of PEG in the MS medium. The objective was to develop an effective system for in vitro screening of potato for drought tolerance.

The MS (Murashige and Skoog 1962) medium with 30 gl^{-1} sucrose and no hormones was supplemented with four levels (0.000, 0.006, 0.012 and 0.018 M) of PEG (Mol wt 8000, SIGMA, USA). The pH of the media was adjusted to 5.7±1.0, and media solidified with 7 gl^{-1} agar (Kanto Chemical Co. Inc., Japan). The water potential of these four media was measured. The media were poured in test tubes (150 x 25 mm), to generate seven treatments (Table 3), four of which were single-layer media (20 ml per test tube), with four levels of PEG, respectively, and three were double-layer media with 0.006, 0.012 and 0.018 M of PEG, respectively, in the lower layer medium (10 ml of per test tube) and 0.000 M PEG in the upper layer medium (10 ml per test tube). In test tubes with double-layer media, the medium without PEG was poured when the lower layer medium with PEG had solidified. The tubes were closed with cotton plugs, autoclaved and used for culturing. The test tubes were taken out of the autoclave after the media had solidified to avoid any mixing of the medium of upper layer with that of the lower layer. Since the medium in the lower layer was heavier than that of the upper layer, there was little mixing of media of two layers, and these were visibly distinct.

Well sprouted microtubers (each of 0.3-0.5 g, with a 0.5-1.0 cm long sprout) supplied by Kirin Brewery Co. Ltd. (Japan) were used as explants. These were washed with liquid detergent in running water for 1-2 min, disinfected with 0.1% mercuric chloride for 3 min, rinsed with autoclaved water three times and cultured aseptically (1 microtuber/test tube). The cultures were incubated at 24±1°C, under 16 h photoperiod/day providing a photon flux of 80-100 μM $m^{-2}s^{-1}$ from white fluorescent tubes

When cultures were 90 days old (without sub-culturing) and fully grown having stout stems and broad leaves in the control treatment (i.e. MS medium without PEG), data were recorded for various shoot and root characters. At the time of observation, the plantlets, in general, had just started showing signs of senescence (browning of leaf edges and/or shoot tips) and were without any microtuber formation. Plantlets were taken out of the tubes, and number of nodal roots (those originating directly from the basal stem nodes) were counted. Shoot was cut from the roots, and plantlet height was measured as the length of the main stem from base to the tip. Foliage (stems and leaves) was cut into pieces and weighed. Roots were washed to remove any sticking medium and preserved in 70% alcohol, and later measured for root length, root diameter, root volume and root-dry weight. The former three characters were recorded using an image analysis system (WinRhizo, Regent Instruments Inc., Canada) and root dry weight was determined after oven-drying the samples to a constant weight at 80°C.

The experiment was conducted in a completely randomized two factor (3 genotypes × 7 media treatments) factorial design with 8 replications. As explant growth was not normal in some replications, the effective number of replications used for data recording was reduced to six. Data were analyzed using the software CPCS1 (Punjab Agricultural University, Ludhiana, India).

MS medium with no PEG had a water potential of -0.80 MPa and it decreased to the lowest level of -1.40 Mpa in the medium with 0.018 M of PEG. The decrease in the water potential of the medium with increase in the level of PEG, expectedly, showed that PEG induced water-stress in the medium. As reported for field conditions (Deblonde and Ledent 2001; Tourneux et al. 2003; Lahlou and Ledent 2005), induced water-stress had adverse effect on the expression of various charcters under in vitro conditions also. This confirms our earlier reports (Gopal and Iwama 2007; Gopal et al. 2008) that in vitro system can be used as an alternative to field evaluations for studying the general effect of water-stress on plant growth and development

Analysis of variance showed that mean squares of various characters were significant due to medium, genotypes as well as medium x genotype interaction except that genotypes did not differ for number of nodes, and medium x genotype interaction was not significant for number of roots. The number of roots decreased with increase in the level of PEG in both single-layer and double-layer media. The maximum number of roots (10.5) averaged over genotypes was in the single-layer medium with no PEG and there was no rooting in any genotype with 0.018 M of PEG. Among the three genotypes, the maximum number of roots averaged over media was in IWA-1 (7.02/plantlet) which was significantly higher that that of in IWA-3 (3.19) and IWA-5 (2.95), and the latter two genotypes did not differ from each other significantly.

The results for other characters for which medium x genotype interactions were significant are presented in Table 1. No consistent pattern of genotypic differences was observed for the performance of various foliage characters in different media tested except that all genotypes were at par on single layer medium with 0.012 and 0.018 M PEG on which plantlets grew too little. Similarly there was no consistent pattern of genotypic differences for root diameter in various media. However, for other root characters i.e. total root length, root volume as well as root dry weight, in double-layer media with 0.006 and 0.012 M PEG in the lower layer, IWA-1 was significantly better than both IWA-3 and IWA-5, which were at par. These differences were sharper in medium with 0.012 M PEG in the lower layer. IWA-1 had longer roots with higher root dry weight than IWA-3 and IWA-5 even in the single layer medium without PEG, but the differences were not as sharp as in the above mentioned double-layer media. Further, such genotypic differences were not observed for root volume in the single-layer medium without PEG. The genotypic differences for these characters were in general not significant in single-layer media with 0.012 and 0.018 M PEG and in double-layer medium with 0.018 M PEG in the lower layer. In single-layer medium with 0.006 M PEG, IWA-1 had higher root dry weight than both IWA-3 and IWA-5, which were at par, but such genotypic pattern was not observed for root length and root volume. In double-layer medium with same level of PEG in the lower layer as in the single-layer medium, root growth was higher than that of in the single-layer medium.

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Table 1. Effect of different media on the performance of genotypes for various characters

		Single-layer medium with PEG				*Double layer medium with no PEG in upper layer and lower layer with PEG*		
Character	*Genotype*	*0.000 M*	*0.006 M*	*0.012 M*	*0.018 M*	*0.006 M*	*0.012 M*	*0.018 M*
Plant height (cm)	IWA-1	10.5 a	9.2 b	4.2 hi	1.0 j	7.9 cd	7.8 cd	6.2 fg
	IWA-3	11.6 a	7.5 cde	3.6 i	0.6 j	8.3 bc	8.2 bc	7.3 cdef
	IWA-5	8.0 b	6.5 ef	3.0 i	0.8 j	6.8 def	5.1 gh	3.3 i
No of nodes/main stem	IWA-1	11.7 a	11.0 a	6.3 efgh	3.2 j	8.0 cde	8.3 bcd	7.2 cdef
	IWA-3	11.8 a	7.0 cdefg	5.2 ghi	3.8 ij	10.0 ab	8.5 bc	7.7 cde
	IWA-5	11.5 a	6.5 defgh	8.2 bcde	4.7 hij	11.5 a	8.5 bc	5.3 fghi
Internodes length(cm)	IWA-1	0.93 abc	0.84abcde	0.70 cdef	0.34 gh	1.01 ab	0.94 ab	0.87 bcd
	IWA-3	0.98 ab	1.16 a	0.69 def	0.16 h	0.83 bcde	0.97 ab	0.99 ab
	IWA-5	0.95 ab	1.05 ab	0.50 fg	0.17 h	0.62 ef	0.70 cdef	0.63 ef
Foliage Fresh wt(g/plantlet)	IWA-1	1.24 a	0.51 cd	0.09 efg	0.01 fg	0.75 bc	0.28 de	0.13 efg
	IWA-3	0.64 bc	0.11 efg	0.03 fg	0.01 fg	0.50 cd	0.27 def	0.13 efg
	IWA-5	0.81 b	0.266 def	0.041 g	0.009 fg	0.290 de	0.284 de	0.06 efg
Total root length (cm)	IWA-1	671 a	357 cd	69 g	4 h	423 bc	320 de	124 fg
	IWA-3	471 b	270 de	0 h	0 h	140 fg	127 fg	104 fg
	IWA-5	485 b	263 e	0 h	0 h	127 fg	165 f	47 gh
Av. root diameter(mm)	IWA-1	0.28 j	0.39 bcd	0.36 cde	0.31 ghij	0.32 efghij	0.37 bcde	0.35 defghi
	IWA-3	0.32 fghij	0.45 a	0.35defgh	-	0.35 defgh	0.41 abc	0.35 defg
	IWA-5	0.29 ij	0.41 ab	0.33 efghij	-	0.30 hij	0.39 bcd	0.36 cdef
Root volume(cm^3/plantlet)	IWA-1	0.42 ab	0.42 ab	0.07 ghij	0.00 ij	0.39 abcd	0.35 abcd	0.12 fgh
	IWA-3	0.36 abc	0.44 a	0.00 j	0.00 j	0.18 ef	0.15 fg	0.12 fgh
	IWA-5	0.34 bcd	0.31 cd	0.00 j	0.00 j	0.09 fghi	0.26 de	0.05 hij
Root dry wt (mg/plantlet)	IWA-1	49.5 a	49.5 a	8.2 fg	0.0 g	33.7 bc	36.8 b	21.7 cde
	IWA-3	33.8 bc	30.7 bcd	0.0 g	0.0 g	14.4 ef	9.8 efg	12.5 ef
	IWA-5	34.6 b	31.7 bc	0.0 g	0.0 g	21.2 efg	12.0 ef	7.3 fg

Figures with common letters are not significantly different by LSD at P≤0.05; - means no rooting

The results showed that the pattern of genotypic differences for root growth (root length, root volume and root dry weight) in three genotypes IWA-1, IWA-3 and IWA-5 in vitro on double-layer media with 0.006 and 0.012 M PEG in the lower layer, was identical to their known root dry weight under field conditions. Out of these two media, the one with 0.012 M PEG was better as the genotypic differences were more distinct on this. This was due to much higher root growth in IWA-1 than IWA-3 and IWA-5 in the medium with lower layer of 0.012 M PEG than that of 0.006 M PEG. This shows that as under field conditions, IWA-1 had higher root penetration ability into hard layer even under in vitro conditions. This medium was also better than the control single-layer medium with no PEG because the differences were larger on the former. In the latter medium genotypic differences for root volume were not significant. The results thus conclusively showed that double-layer medium with no PEG in the upper layer and 0.012 M PEG in the lower layer can be a good alternative to field evaluation of potato genotypes for root growth.

The root growth was drastically reduced in single-layer media with more than 0.012 and 0.018 M PEG and double-layer medium with 0.018 M PEG in the lower layer, and genotypic differences for root growth were not significant (Table 1). This shows that in single-layer media with ≥ 0.012 M PEG roots growth was equally inhibited in all genotypes, and it was so in the double-layer medium with 0.018 M PEG in the lower layer where roots developed in the upper layer medium without PEG were equally inhibited in all genotypes in the lower layer. Hence, water-stress only to a limited extent could be used for differentiating the potato genotypes for root growth. This confirms our earlier report that when water-stress crossed a particular limit, even IWA-1 was not able to grow (Gopal and Iwama 2007; Gopal et al. 2008). The present study, however, further showed that this would be applicable to use of both single-layer and double-layer media although in the latter a higher level of water-stress could be tolerated due to intial growth of the roots in the upper layer with little water-stress (no PEG). Due to this, IWA-1 selected for higher root dry weight under field conditions could be better differentiated from IWA-3 and IWA-5 in vitro on double-layer medium than single-layer medium.

The results showed that root length, root volume as well root dry weight followed similar pattern of genotypic differences on the identified two best double-layer media (i.e. with 0.012 and 0.018 M PEG in the lower layer) for in vitro screening of potato genotypes for roots growth. Thus any of these characters can be used for measuring the root growth. Root dry matter which is easier to record and reflects the overall root amount should be a good choice. Root diameter as well as none of the foliage characters followed the pattern of the root growth observed in various genotypes; hence none of these can be used as indicators of roots growth.

Root fresh weight in vitro (Morpurgo 1991) as well as in vivo (Iwama et al. 1982) is reported to have a positive correlation with tuber yield in vivo. Under field conditions, larger root dry weight delays leaf senescence and thus prolongs tuber bulking (Iwama et al. 1982). IWA-1 having the higher root dry weight is very late in maturity. IWA-3 is late in maturity than IWA-5, which is of medium-late maturity. These genotypes represented the different combinations of root-dry weight and foliage maturity. A genotype with high or medium root dry weight and early maturity perhaps is too difficult to find (Iwama et al. 1981). The observed relationship in these genotypes of variable foliage maturity for root growth in field and on specific in vitro media thus is due to the inherent higher drought-tolerance ability of IWA-1 compared with IWA-3 and IWA-5 that had been confirmed based on well-conducted field trials repeated over years. Unfortunately such field evaluations are available for few genotypes, thereby limiting the number of genotypes that could be used for this study. Long term studies are now being made by us to evaluate a larger number of genotypes for root dry weight under varying greenhouse, field and in vitro conditions to further refine and define methods suitable for screening potato for drought-tolerance.

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Virology

EFFECTIVENESS OF PARAFFINIC MINERAL OIL, INSECTICIDES AND VEGETAL OIL TO CONTROL POTATO VIRUS Y (PVY) SPREAD IN POTATO SEEDS MULTIPLICATION FIELDS.

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Abstract.

Trials aiming at defining the effectiveness of different fighting schemes against the potato virus Y spread in potato seeds fields were led in the Walloon Region (Belgium) between 1998 and 2006. In the 1998/2000 trial, the use of paraffinic mineral oil applied alone showed a reduction of infection of 68.5% in average when compared to a control without any protection, while the effectiveness of a classical scheme combining mineral oils and insecticide reduced the PVY infection of 74.8%. In the same trial, in 1999, we tested an insecticide used alone, this strategy reducing the infection of 8.4% only. In 2006, an other trial tested the effectiveness of two insecticides applied alone (Sumi alpha and Plenum) compared to fighting strategies using paraffinic mineral oil alone or a combination of paraffinic mineral oil and Plenum : the results showed clearly, once again, that insecticides can not reduce significantly the level of PVY infection when applied alone, while the use of paraffinic mineral oil, applied alone or in combination with Plenum, strongly and significantly reduced the level of infection (58% for paraffinic mineral oil only and 51% for the combination).

In 2001, the effectiveness of a vegetal oil against PVY dissimination in potato seeds field was compared to the application of paraffinic mineral oil: the results showed that both gave a significative reduction of infection but the effectiveness was better with paraffinic mineral oil (50.3%) than with vegetal oil (27.4% when applied at 15l/ha, 17.3% when applied at 7l/ha). According to the fact, often reported, that insecticides are not usefull to reduce the contaminations by non persistant viruses , all these results show that the chemical fight against PVY infections in potato seeds fields must be based on the use of paraffinic mineral oil and that the frequency of insecticides application could be lowered. Insecticide application for that specific purpose is still too often recommended by the private companies and/or officials.

Introduction.

Potato virus Y represents the most important problem in the potato seeds fields in West Europa and elsewhere. In Belgium, PVY infects between 40 to 80 % of the potato seeds fields and is responsible for most of the seed lots downgrading when controled in laboratory (more than 90%) (Rolot, 2005). This situation comes from the transmission type of the virus, operated on the non persistant way. It is a virus which can be easily acquired and transmitted by a large spectra of aphids species for which the potato is not necessarily an host: quick acquisition when making their feeding probe on an infected plant, no latent period, the aphids being able to transmit the acquired virus just after the feeding probe (Bradley and Rideout, 1953). The climatic change that is running in West Europe (milder winters that encourages the survival rate of parthenogenetically forms and warmer springs, both determining earlier and more important aphids fly) as well as the change in the PVY strains (necrotic strains) are factors which can make worse the PVY issue in the near future (Lindner & al, 2005, Rolot & al, 2007).

The control of PVY dissimination in potato seeds fields must be based first on cultural practices such as the choice of good seeds lots to be multiplied (lots without or with a very low level of infection), of the field location (isolation, low pressure of aphids), of the variety to be multiplied (resistant ones if the market enables the development of such varieties) and an early roguing.

Pesticides use, such as insecticides and paraffinic mineral oils, remains however a practice considered to be necessary by the majority of the growers in our fields conditions.

In Belgium, PVY control is based on a weekly or even bi-weekly protection scheme with insecticides (one dosis / week) and paraffinic mineral oils (7 l / ha and week).

In the trials set up during these last ten years, we wanted to check the validity of such intensive protection schemes in order to identify the best practice to promote. In the period between 1998 and 2000 we made our first trial in field conditions in order to underline the effect of reduced programs of protection on PVY dissemination at the field scale. A strategy of protection using paraffinic mineral oils alone was compared to strategies using a combination of paraffinic mineral oils and insecticide or using insecticide alone. In 2001 we made another trial testing the effectiveness of a vegetal oil compared to the application of paraffinic mineral oil and, in 2006, we compared the effectiveness of two insecticides applied alone for the reduction of PVY infection to the effectiveness given by an application of parraffinic mineral oil.

Material and method.

The characteristics of the different trials are shown in the table 1.

Table 1. Characteristics of the trials performed in 1998/2000, 2001 and 2006.

Year	Location	Tested objects (treatments)	Trial design and characteristics	Factors	Measured parameters	Samples
1998, 1999, 2000	Ardenne Condroz Famenne	1 Control (no protection) 2 Paraffinic mineral oil (6l / week / ha + insecticide Mavrik B (0.6l / ha / week) 3 Paraffinic mineral oil alone (6l / ha / week) 4 Insecticide alone Mavrik B (0.6l / ha / week)	Randomized blocks with systematic control Big plots (50mx13m) Homogeneous lots of Bintje seeds, 1,25% PVY in 1998, 1.3% PVY in 1999 and 4% PVY in 2000	YearxLocation (9 - random) Treatment (2 - fix) Number of observations= 3	1 Reduction of PVY infection compared to the control (%) 2 Number of winged aphids in the foliage (n) 3 Number of apterous aphids in the foliage (n)	1 240 tubers / plot, DAS-ELISA analysis 2 3 Observation on 20 complete leaves, 6 times
2001	Ardenne	1 Control (no protection) 2 Paraffinic mineral oil alone (6l / ha / week) 3 Vegetal oil alone (15l / ha / week) 4 Vegetal oil alone (7l / ha / week)	Randomized blocks Plots: 4 lines of 30 plants (healthy minitubers, variety Bintje) with 1 infected tuber in the middle of the two central lines	Block (4 - random) Treatment (4 - fix)	1 Reduction of PVY infection compared to the control (%)	1 60 tubers / plot (central lines), DAS-ELISA analysis
2006	Ardenne	1 Control (no protection) 2 Insecticide Sumi alpha alone (0.2l / ha / 2 weeks) 3 Insecticide Plenum alone (300g / ha / 2 weeks) 4 Paraffinic mineral oil alone (6l / ha / week) 5 Paraffinic mineral oil (6l / ha / week) + Insecticide Plenum (300 g / ha / 2 weeks)	Randomized blocks Plots: 4 lines of 30 plants (healthy minitubers, variety Bintje) with 1 infected tuber in the middle of the two central lines	Block (4 -random) Treatment (5-fix)	1 Reduction of PVY infection compared to the control (%) 2 Number of winged aphids in foliage (log(n+1)) 3 Number of apterous aphids in the foliage (log (n+1))	1 60 tubers / plot (central lines), DAS-ELISA analysis 2 3 Observation on 10 complete leaves, 8 times

The products used were:

-1998-1999-2000:	paraffinic mineral oil:	BIOPALINE 1535 (826g/l)
	insecticide :	MAVRIK B (thiomethon 200g/l + fluvalinate 72g/l)
-2001:	paraffinic mineral oil:	BIOPALINE 1535 (826g/l)
	vegetal oil :	TELMION (Rape Seed Oil 85%)
-2006:	paraffinic mineral oil:	PROTEX OIL (850g/l)
	insecticides :	SUMI ALPHA (Esfenvalerate, 25g/l), PLENUM (Pymetrozine,500g/kg)

Treatment effectiveness against the spread of PVY was measured by the reduction of PVY infection (%) obtained in the treated plots on tubers samples, compared to the one measured in the control (not protected) plots:

$Y = [1-a/b] * 100$ where : Y= PVY infection reduction rate (PVY effectiveness),
a= PVY infection in the treated object
b= average of PVY infection in control plots

Results

1. 1998-1999-2000 trial.

1.1 Treatments efficiency against PVY dissimination.

Table 2. Measured PVY infection rate (mean ± standard deviation, %) in tuber samples for the different treatments, years and locations

Treatment	Location	1998	1999	2000
Control	Ardenne	11.7 ± 1.5	20.3 ± 4.7	41.0 ± 6.2
	Famenne	5.7 ± 1.1	27.0 ± 3.5	44.3 ± 6.7
	Condroz	19.7 ± 3.3	20.0 ± 5.2	47.7 ± 3.1
Oil + Insecticide	Ardenne	4.7 ± 0.6	4.7 ± 1.1	4.7 ± 1.1
	Famenne	0.7 ± 0.6	10.3 ± 1.5	7.0 ± 3.6
	Condroz	6.7 ± 1.5	6.0 ± 1.0	8.7 ± 2.5
Oil	Ardenne	4.3 ± 0.6	8.7 ± 1.1	8.3 ± 3.8
	Famenne	1.2 ± 0.6	8.7 ± 0.6	15.0 ± 3.0
	Condroz	5.0 ± 1.0	8.7 ±2.3	8.7 ± 1.5
Insecticide	Ardenne	-	15.3 ± 1.1	-
	Famenne	-	30.7 ± 7.6	-
	Condroz	-	15.3 ±2.3	-

A mixed, partially nested, 3 ways ANOVA was performed considering the factors "Treatments" as fix, "Year x Location" as random and "Block" as random and nested to the "Year x Location" factor. "Treatments" was tested against the interaction "Treatment" x "Year x Location".

The results showed that, globally, there wasn't a significative difference between the treatments ($F(1,8)=3.07$; $p=0.12$). The addition of the insecticide Mavrik B in the protection scheme does not seem to increase the protection level against PVY spread.

However, as we are at the limit of a marginally significative effect ($p \leq 0,1$), we performed another analysis considering each year independently (two ways ANOVA with location and treatment as independent factors, the factor “treatment” being tested against the residual error term). The results highlighted a significant effect of the treatment when the insecticide is combined with the mineral oil, but only in some years and some locations (1999 Ardenne and 2000 Famenne) (Fig. 1). These results underlined that, in some conditions, the complementary use of the insecticide Mavrik B has given a better level of protection.

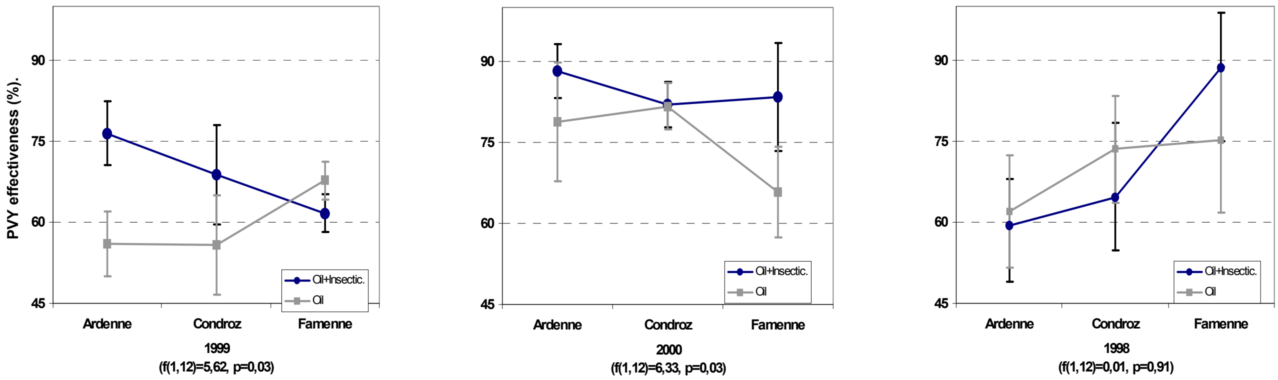

Fig 1. Trial 1998/2000: treatments effectiveness year by year, in each location

In 1999, and only in this year, we introduced in the trial a modality in which the plots were treated only with the insecticide Mavrik B. The infection results proved that the insecticide doesn’t give a protection level as high as the one given by the mineral oil: the reduction of PVY infection obtaind with the insecticide applied alone was only of 8.9% when the one obtained with the mineral oil was of 61.1%.

1.2 Treatment effectiveness on the presence of apterous and alate aphids in the crop foliage.

The analysis of the aphids abundance observed in the plots was performed by a two ways ANOVA (« Year x Location »- random - 9 levels and « treatment » - fix - 3 levels).

The analysis showed that the treatments gave a significative and different effect on aphids abundance in the foliage, and this on the alate aphids ($f(2,16)=7,70$, $p=0,004$) as well as on the apterous aphids ($f(2,16)=8,18$, $p=0,003$). It was the used insecticide that gave this effect: Mavrik B prevented the development of the apterous populations in the crop and reduced significantly the presence of alates. Newman and Keuls test ($\alpha=0,05$) classifyed the treatment using the insecticide (mineral oil + insecticide) and the treatments without insecticide (control and mineral oil only) in two separate groups.
The insecticide effect on the abundance of the aphids populations in the foliage is clearly shown in figure 2. However, the insecticide doesn’t determine the same level of efficiency in the two populations types: if Mavrik B is particularly effective against the apterous population, it seems evident that it doesn’t give the same level of protection against the alate population.

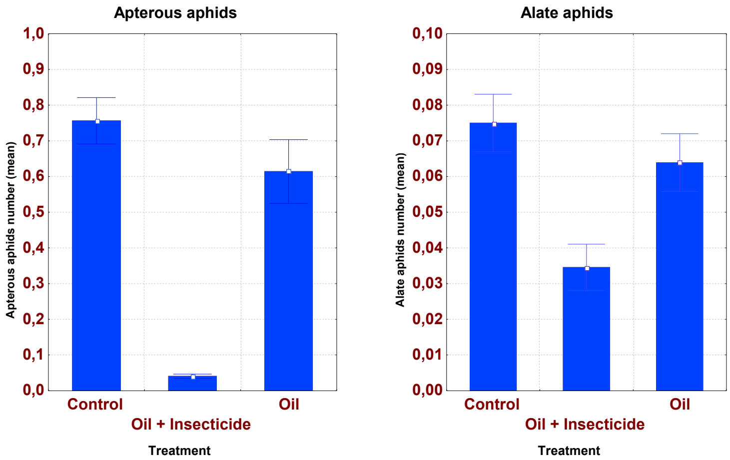

Fig 2. Trial 1998/2000: treatment effect on the presence of apterous and alate aphids in the foliage

2. 2006 trial.

2.1 Treatments efficiency against PVY dissimination.

Table 3 ***Measured PVY infection rate (mean ± standard deviation) in tuber samples for the different treatments.***

Treatment/Object	PVY(%) ± standard deviation
Control	60.75 ± 18.55
Sumi Alpha	56.75 ± 14.34
Plenum	60.75 ± 4.19
Oil	25.00 ± 13.73
Oil + Plenum	29.75 ± 7.54

The statistical analysis (ANOVA, 2 ways, « treatments » - fix - 4 levels, « blocks » - random - 4 levels) showed a significant effect of the applied treatment on the reduction of PVY spread in the field ($F(3,9)=13.61$, $p=0.001$).

Besides the results showing clearly the differences in effectiveness, Newman & Keuls test (α=0,05) classifies the treatments in two different groups, the first including the treatments in which the insecticide was applied alone (Sumi alpha, Plenum) and the second the treatments in which the mineral oil was used, alone or with Plenum.

In average, the application of Sumi alpha reduced the level of infection by 6,5% whereas the effectiveness of Plenum application was 0%. However these two values were not statistically different.

On the other hand, the application of mineral oil alone reduced the infection by 58% and the effectiveness of mineral oil + Plenum was of 51%, these two values were not statistically different

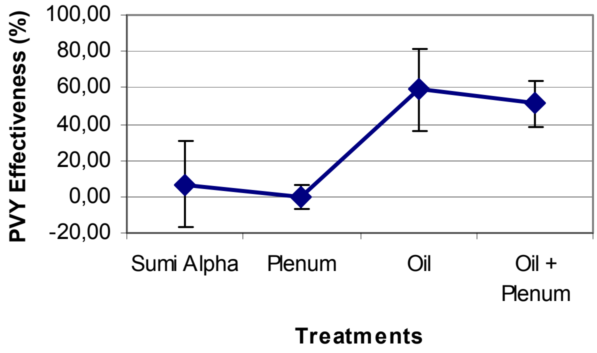

Fig 3. Trial 2006: treatments effectiveness for the reduction of PVY infection

2.2 Treatment effectiveness on the presence of apterous and alate aphids in the crop foliage

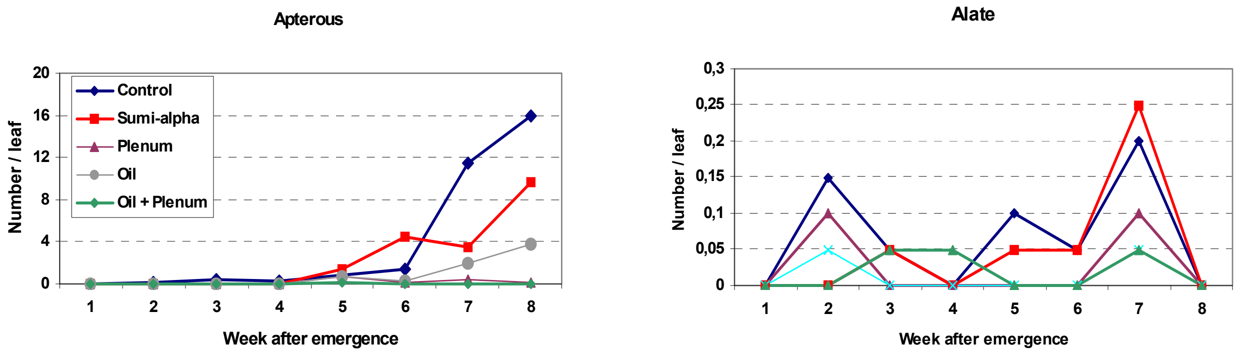

Fig 4. Trial 2006: observed number of alate and apterous aphids (mean) in the foliage, for the different treatments during the season

If figure 4 shows clearly an effect of the applied treatment on the abundance of apterous aphids in the crop, it does not show any effect on the presence of alates.

Our observations have shown that Sumi alpha didn't control efficiently the development of apterous colonies in the foliage and this was particularly clear for Aphis nasturtii of which the abundance was high, also in the control plots and in the plots treated with the mineral oil . At the opposite, Plenum had a very good control on the apterous and especially on A. nasturtii. Nevertheless, this good aphicide action didn't have a good impact against PVY dissemination. This underlines that the apterous are not significantly involved in PVY spread. The better results obtained with the application of mineral oil in spite of a higher presence of apterous in the foliage, is also an evidence of the poor action of the apterous on PVY dispersal : the poor mobility of the apterous between plants impedes the dissimination of PVY in the crop. So, PVY, that is rather disseminated by the alates aphids, is not so well controled by the insecticides applications.

3. 2001 trial.

3.1 Treatments efficiency against PVY dissemination.

Table 4. Measured PVY infection rate (mean ± standard deviation) in tuber samples for the different treatments.

Treatment/Object	PVY(%) ± standard deviation
Control	69,17 ± 1,34
Mineral oil (6l)	34,37 ± 7,43
Vegatal oil (7l)	57,22 ± 9,16
Vegetal oil (15l)	50,22 ± 6,14

The statistical analysis (two ways ANOVA , « treatments » - fix - 3 levels, « block » - random - 4 levels) shows a significant effect of the different treatments on the reduction of PVY dissemination in the crop (F(2,6)=8,38, p=0,018).

The effectiveness of the application of mineral oil (reduction of infection =50,3%) was statistically better than the one obtained with the application of vegetal oil (27,4% (15l), 17,3% (7l)).

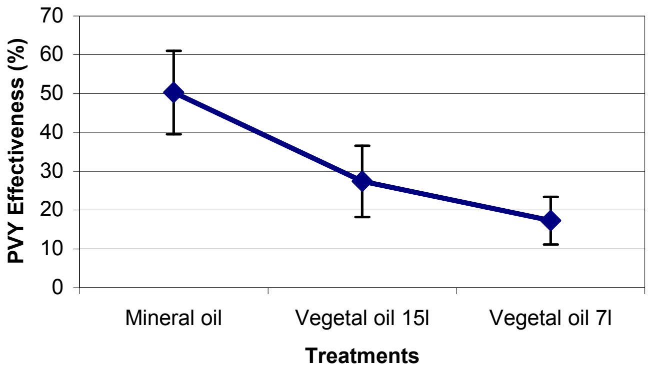

Fig 5. Trial 2001: treatments effectiveness for the reduction of PVY infection.

Conclusions.

These trials, performed in Belgium during these last ten years, have clearly shown that paraffinic mineral oils must be used in priority in the potato seeds multiplication fields when the Potato virus Y is the major sanitary problem.

In the conditions in which our trials were led (no specific cultural measures against the PVY dissemination, for example no roguing), we have shown that the sole use of paraffinic mineral oil at a dosis of 6l/ha/week can reduce the infections by about 60% in tubers while the insecticides can not or can be only of a little help in some conditions.

These results must be used as arguments to convince the growers for a reduction in the use of insecticides, for example by decreasing the frequency of insecticide applications in the potato seeds multiplication fields.

However, the reduction of insecticide applications must take into account the presence of the Potato leaf roll virus (PLRV), as the protection scheme against the dissimination of this virus must be organised around the use of insecticides. In Belgium, PLRV is, at present, not common, and besides, PLRV infection has been traced in all the samples. Our results did not shown any particular infection increase without the use of the insecticides.

Regarding the effectiveness of the vegetal oil "Telmion", we showed that the application of such a product can reduce the level of infection but the protection level was not so high than the one obtained with the mineral oil and this even with more than a double dose of vegetal oil.

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DETECTION AND MANAGEMENT OF TOBACCO RATTLE VIRUS

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Tobacco rattle virus (TRV) causes spraing or corky-ringspot in potato tubers. It is transmitted by free-living trichodorid nematodes and has an extensive weed host range. The virus and its vectors are found in lighter soils. Few methods are available to control TRV infection, e.g. inherent resistance and nematicides. Methods of detection are laborious, relying on counting trichodorids or on bait tests. SCRI has demonstrated the detection of the virus in roots of weeds within fields as a 'bioassay' to 'map' the distribution of the virus allowing growers to target risk areas for nematicide or crop avoidance. Results of sampling and virus distributions in fields are presented and possible control strategies discussed.

ADVANCES IN POTATO MOP-TOP VIRUS RESEARCH

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Presentation will include studies on virus cell-to-cell and long distance movement, the role and function of virus movement proteins and implication of recent data for vector transmission.

DISSEMINATION OF POTATO VIRUS Y (PVY) ISOLATES UNDER FIELD CONDITIONS

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Keywords: Potyvirus Y (PVY), PVY isolate, ELISA test, variety assessment

In the last few years, many new isolates of PVY (potyvirus Y) appeared in the potato fields in Switzerland. New recombinations have been revealed by PCR methods (Rigotti and Gugerli, 2004). The dissemination rate and the sensitivity of the cultivars changed fundamentally. Switzerland has intensive trade exchange of potato seed with different European areas and countries. These exchanges accelerate the dissemination of the different predominant PVY types. Early nineties, first PVY^{NTN} potato tuber ringspot disease symptoms on new breeding variety Lido have been observed in Switzerland. In the beginning of this millenary, PVY^{Wilga} has been detected.

The dissemination of six different PVY isolates on six different cultivars has been studied under field conditions. The rectangular small potato plots, 800 seed tubers each, were separated by twelve meters bands of oat. This cereal was sown at same time than potatoes but since oat grew before potatoes, it could help reducing attractiveness for wing aphides and to move between plots at the beginning of the growing season. In each variety plot, 4 % of a single PVY isolate infected potato plants were inserted. For the control of the dissemination of PVY by wing aphids from one plot to another, PVY^{O} and PVY^{N} antibody ELISA tests have been conducted separately.

Under these field conditions, PVY ring necrosis symptoms on tubers have been detected in promising new varieties. Dissemination of different PVY isolates from one plot to another, isolated with oat bands, was very low in 2005 and 2006. The cultivar Lady Christl was resistant to all PVY isolate infections; Bintje and Charlotte were sensitive to PVY and very sensitive to PVY^{NTN} and PVY^{Wilga} isolates. Charlotte was very sensitive to PVY^{N} isolates in 2006. Spread of PVY^{O} and PVY^{N} was lower then PVY^{NTN} and PVY^{Wilga} isolates. Ring necroses on tubers were mostly observed on Nicola and rarely on Bintje, Desiree or Charlotte.

Some new PVY isolates were responsible for PVY ring necroses on tubers for sensitive varieties like Nicola, Ditta, Erntestolz, Verdi, Juliette, Derby, Arielle and Amandine. The sensitivity of cultivars to PVY is variable and differs according to the necroses tuber-sensitivity.

Introduction

Switzerland is a small mountain country with 7 million inhabitants and a large mountain area. Arable land represents only one quarter of the total surface (40'000 km^2). Around 30 European potato varieties are cultivated on these 12'000 ha with an average yield of 38 to 42t/ha. The seed production covers about 1500 ha. Located in the centre of Europe, Switzerland is not a member of the European Union.

In Switzerland the main potato production area is located on the Plateau, a long piece of land, 400 to 800 m above sea level between the Jura (a low-altitude mountain range) in the North and the Alps in the South. Several kind of wing aphid populations, as virus vector, appears early in spring at the same time as potato plant emergence. In Switzerland, the wing aphid development is very favourable, much more then in northern part of Europe with constant cool, windy areas. For economical and rational evolution reasons, the seed production in Switzerland has been transferred from isolated high altitude above sea level areas to high-density potato growing areas on the Swiss plateau. The renewal rate of seed potatoes is high with about 70%. Usually, 10 to 15 % of the seeds, mostly basic seed category, are imported from Holland, Germany, or Bretagne (France), and exceptionally from North of France, Belgium or Austria. Because of the regular potato import of variable origin and the fast dissemination rate of the virus, Switzerland is characterized as a collector area of many types of potyvirus. Soft viruses such as PVS, PVX, PVA and PVM, but also severs viruses such as PLRV and many types of potyvirus Y isolates.

Since 1995, 95% of the sever virus infections have been caused by PVY. In the last few years, many new isolates of PVY (potyvirus Y) were characterized on potato fields in Switzerland. New recombinations have been revealed by PCR methods (Rigotti and Gugerli, 2004). The dissemination rapidity of the old PVY isolates and the sensitivity of the cultivars changed fundamentally. One of the reasons is that Switzerland has regular intensive trade exchange of potato seed with different European areas and countries. These exchanges accelerate the dissemination of the diverse predominant PVY types originally found in different parts of Europe. Early nineties, the first PVY^{NTN} potato tuber ringspot disease symptoms on the new breeding variety Lido have been observed. The real dissemination of ringspot types started in 2000 with the cultivars Nicola, Erntestolz, Ditta and Hermes. At the beginning of this millenary, PVY^{Wilga} has been detected.

Method

The dissemination of six different PVY isolates on six potato cultivars (Bintje, Charlotte, Desiree, Lady Christl, Marlen, Nicola) have been carried out under field conditions. Sixteen plots of 200 m^2 containing each a total of 800 healthy seed tubers of 6 cultivars were separated by twelve meters bands of oat to reduce the attractiveness for wing aphides to move between plots at the beginning of the growing season. In every plot and for every cultivar two rows of 50 tubers each were grown. In each plot 4% of PVY infected plants of the cultivar Nicola were planted on the 15^{th} and on the 35^{th} plant-position of every 50 plant rows of a single PVY isolate and harvested one week before the end of the trial. PVY isolates were produced under greenhouse conditions (each PVY isolate in a separate compartment) one year before the field trial. The infection with the different PVY isolates was made manually by abrasion of 5 leafs when the potato plants reached 30-50 cm high. In the control plot Nicola plants were treated with water. From each cultivar and plot, 200 tubers were harvested. The dormancy of these tubers was then broken by Rindite. Five weeks later, control of the dissemination of PVY by wing aphids between plots was done conducting PVY^O and PVY^N antibody ELISA tests separately on each tuber. A second sample of 200 tubers was also controlled visually for ring spot symptoms just after harvest and 2 months after stocking.
Using similar oats band isolation design, two other experiments were carried out. First, the yield of healthy Nicola plants was compared with Nicola plants infected by different PVY isolates. Secondly, PVY ring spot necrosis sensitivity on tubers was studied for all promising varieties present in the Swiss official cultivar field trials. Since five years, this study has been part of the variety assessment in Switzerland. In each no infected variety plot, 4 % PVY^{NTN} infected potato plants (Nicola and Erntestolz) were inserted. These infected plants were harvested one week before the other plants in the plots. The yielded tubers of promising varieties are controlled visually.

Results

The cultivar Lady Christl was surprisingly highly resistant to all PVY isolate infections; Bintje and Charlotte were sensitive to PVY and very sensitive to PVY^{NTN} and PVY^{Wilga} isolates. Charlotte was very sensitive to PVY^N isolates in 2006. Spread of PVY^{O803} and PVY^{N605} was lower than PVY^{NTN} and PVY^{Wilga} isolates (figure 1). Formerly, Nicola and Desiree were reputed to be well resistant to PVY, but today, these cultivars are sensitive to the new isolates of NTN (necrotic) or $PVY^{Wilga.}$. The isolates of PVY^{O803} and PVY^{NTN854} are very phytotoxic for Nicola and Desiree. Some of the infected plants died before harvest and did not serve as a virus source for the healthy plants during the growing season. The cultivation of vigorous infected plants with the different isolates of PVY is very challenging. The small PVY^{O803} infected plants where rapidly covered by the healthy vigorous plants.
After the harvest, ring necroses on tubers were mostly observed on Nicola and rarely on Bintje, Desiree or Charlotte.
Under field conditions, the spread of diverse PVY isolates between plots was very low in 2005 and 2006 (figure 2). The 12 meters isolation bands of well-established oat between the plots were sufficient to prevent isolates transfer via wing aphids.
The cultivar assessment denotes sensitivity to ring necroses on tubers for sensitive varieties like Nicola, Ditta, Erntestolz, Verdi, Juliette, Derby, Arielle and Amandine. These cultivars should not be selected as recommended varieties for Switzerland. The sensitivity of the cultivars tested in our study is variable and differs from the necroses tuber-sensitivity.

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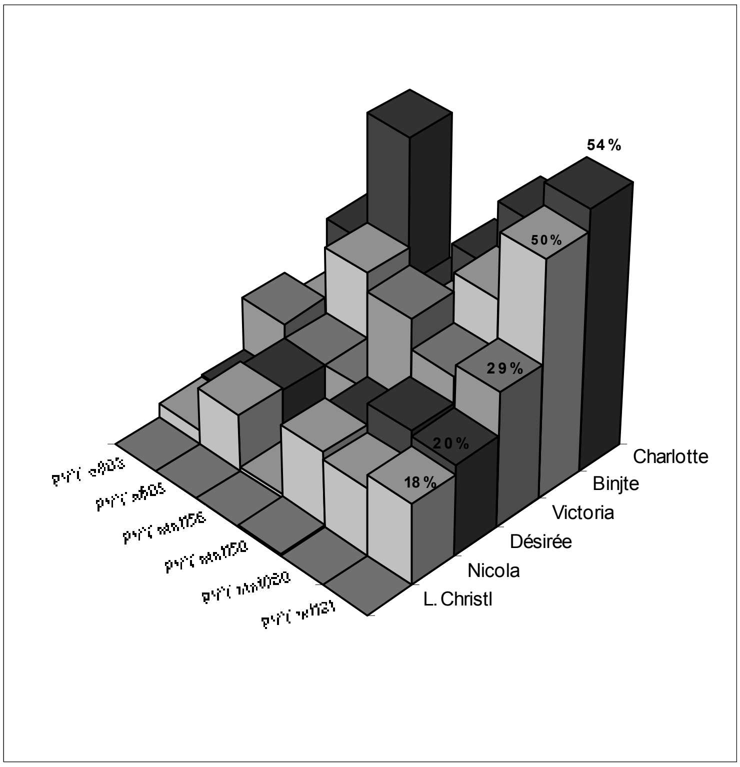

Figure 1. Results of ELISA test on tubers growing under field conditions in 2006. The six cultivars have different sensitivities to the Potyvirus Y isolates. Lady Christl was surprisingly high resistant. While Nicola and Desiree were reputed to have good PVY resistances, these cultivars are now sensitive to new PVY isolates. Charlotte and Bintje are very sensitive to PVY.

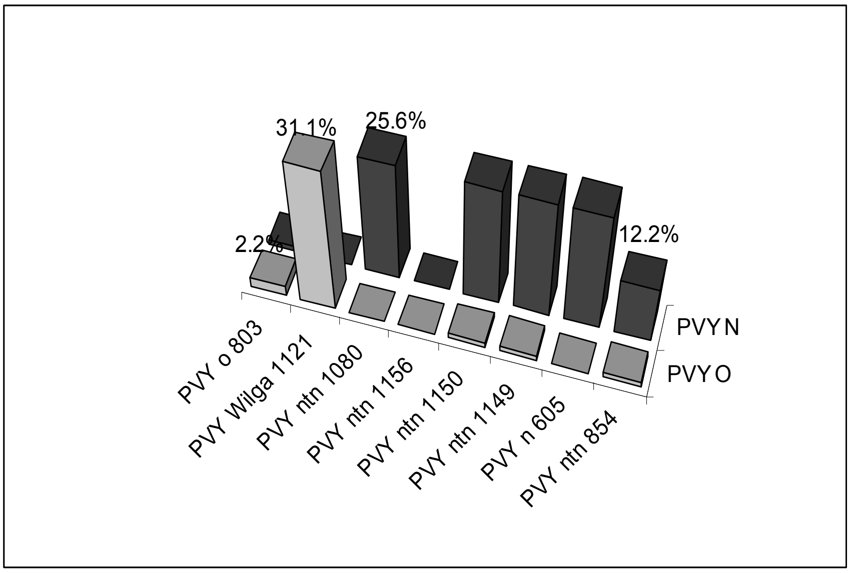

Figure 2. The ELISA test enables to distinguish PVY-o and –n isolates. ELISA test results of the trials under field conditions show that contaminations from PVYN plots to PVYO plots are very low. The separation band of oat between the different plots was sufficient. The contamination level of the new PVY isolates was higher for the Wilga type or for the NTN isolates.

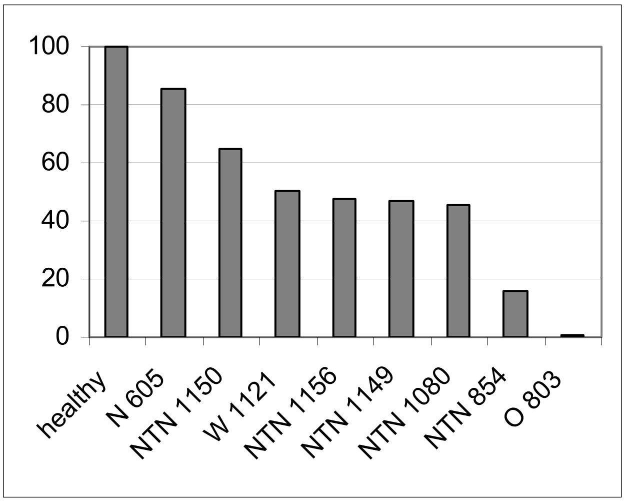

Figure 3. The yield of different isolates of PVY under field conditions was observed in another trial. The yield of the cultivar Nicola was not much affected by the isolate PVY ^{N605}. Most of NTN isolates gave only half yield and 80% of the tubers showed ring necroses. The isolates PVY^{NTN854} and PVY^{O803} were very phototoxic for Nicola and many infected plants died before harvest.

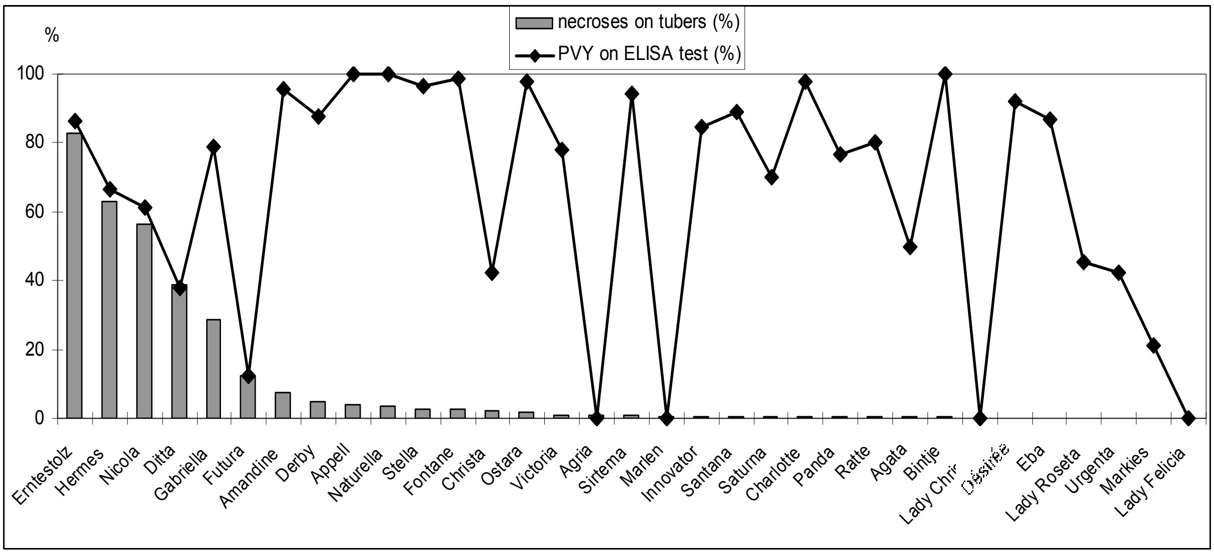

Figure 4. Cultivars with high sensitivity to PVY dissemination are not necessary sensitive to ring necrosis symptoms on the tubers. These results have been obtained in Changins with very high vector pressure and many infected plants of different isolates of PVY^{NTN}.

Phytoplasma & Zebra Chip

ZEBRA CHIP, A NEW POTATO DISEASE IN NORTH AND CENTRAL AMERICA, IS ASSOCIATED WITH THE POTATO PSYLLID

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Zebra chip (ZC) is an important and emerging potato disease that is causing millions of dollars in losses to both potato producers and processors in the southwestern United States, Mexico, and Central America. This disease is characterized by symptoms that develop in fried chips from infected potato tubers and that consist of a striped pattern of necrosis in tuber cross-section. ZC plant symptoms resemble those caused by potato purple top and psyllid yellows diseases. To date, the exact causal agent(s) and/or vectors of ZC are unknown. However, a survey of insects associated with the potato crop in Texas and Mexico indicated that the potato psyllid, *Bactericera cockerelli* (Sulc), was the most common and abundant insect in all of the ZC-affected potato fields. To increase the understanding of the role of the potato psyllid and phytoplasmas in the expression of ZC, controlled psyllid exposure and exclusion experiments using cages were conducted in the greenhouse and field in Washington State and the Lower Rio Grande Valley of Texas. Also, potato plants and tubers exhibiting ZC symptoms were tested for phytoplasmas by PCR. Results indicated that there was a strong association between the potato psyllid and ZC. Plants exposed to psyllids developed typical ZC symptoms in both raw tubers and fried chips. No single potato plant in the cages or greenhouse rooms without psyllids (controls) showed ZC symptoms in raw tubers or fried chips, suggesting that the observed ZC symptoms were due to psyllid damage. No phytoplasmas were detected in plants or tubers with ZC symptoms, suggesting that these pathogens are not involved in this potato disease.

Introduction

Zebra chip (ZC), a new disease of potato, has recently been documented to occur in potato fields throughout southwestern United States, Mexico, and Central America. This damaging potato disease is causing millions of dollars in losses to both potato producers and processors in affected areas, often causing the abandonment of entire potato fields (Goolsby et al. 2007, Munyaneza et al. 2007a,b). ZC is characterized by symptoms that develop in tubers from infected potato plants and that consist of a striped pattern of necrosis in tuber cross-section (Munyaneza et al. 2007a,b). ZC-infected potato plants exhibit a range of plant symptoms that resemble those caused by potato purple top and psyllid yellows diseases (Wallis 1955, Cranshaw 1994, Crosslin et al. 2005, Munyaneza et al. 2006, 2007a,b). Plant symptoms include stunting, chlorosis, swollen internodes and basal cupping of the upper growth accompanied with yellowish or reddish discoloration, proliferation of axillary buds and aerial tubers, browning of the vascular system in below-ground portions of stems, leaf scorching, and early plant decline. ZC symptoms in tubers include enlarged lenticels of the underground stem, collapsed stolons, and brown discoloration of the vascular ring and necrotic flecking of internal tissues and occasionally streaking of the medullary ray tissues. These necrotic symptoms affect the entire tuber from the stem end to the bud end. Chips made from tubers of affected potato plants have a severe dark brown streaking defect, hence the name 'zebra chip', and are not commercially acceptable. Furthermore, tubers affected with ZC generally do not sprout, or if they do, produce hair sprouts or weak plants (Munyaneza et al. 2007a,b).

To date, the exact causal agent(s) and/or vectors of ZC are unknown. However, a survey of insects associated with the potato crop in Texas indicated that the potato psyllid, *Bactericera cockerelli* (Sulc), was the most common and abundant insect in all of the ZC-affected potato fields (Goolsby et al. 2007). In addition, similar observations have been made in Mexico where this disease is very damaging (Munyaneza et al. 2007a). To increase the understanding of the role of the potato psyllid in the expression of ZC, controlled greenhouse and field psyllid exclusion and exposure experiments were conducted in both Washington State and the Lower Rio Grande Valley of Texas in 2006 and 2007 (Munyaneza et al. 2007a,b). In addition, since ZC symptoms resemble those of the potato purple top disease, investigation of potential ZC causal agents was conducted by testing potato plants and tubers exhibiting typical ZC symptoms for the phytoplasmas that cause potato purple top disease.

Materials and Methods

The field experiments were conducted at both USDA-ARS Research Farms in Yakima, WA and Weslaco, TX in 2006 and 2007. Additional experiments were conducted in the greenhouse at the USDA-ARS Wapato, WA in 2006. Certified clean potato seed of four potato chipping varieties (Atlantic, FL1879, FL1833, and FL1867) were used in the studies. In the greenhouse, potted potato plants were exposed to potato psyllids in small greenhouse rooms under controlled conditions; psyllids were excluded in control rooms. In the field, potatoes were hand-planted in small field plots in both Yakima and Weslaco; the plots were covered with cages made of PVC pipes and an insect screen fabric. Treatments in Yakima experiments consisted of cages with or without psyllids from the laboratory-reared colony. In Weslaco experiment, four treatments were involved: caged plants infected with psyllids from the laboratory colony, caged plants infected with Texas field collected psyllids, caged plants without psyllids, and uncaged plants. In the treatment involving laboratory-reared psyllids, the insects used were from a colony that had been established at the USDA-ARS Laboratory at Wapato, WA, since late fall 2005, from psyllids originally collected from a potato field severely affected by ZC in Dalhart, TX; these psyllids had been maintained on potato and eggplant for several generations. Approximately 300 psyllids were introduced in each cage; the insects were released when the plants in the cages were in the pre-bloom stage. Plants were monitored for ZC symptoms. At harvest, potato tubers were checked for ZC symptoms, processed into chips, and fried to check for chip discoloration and ZC symptoms. Due to the resemblance of ZC plant symptoms and those caused by the potato purple top disease, plants and tubers exhibiting typical ZC symptoms were collected and tested for phytoplasmas using a nested polymerase chain reaction (PCR) assay with universal primer pairs P1/P7 and fU5/rU3 (Crosslin et al. 2006). Percentages of plants with ZC symptoms in raw tubers and fried chips were calculated for each treatment and the data were statistically analyzed.

Results and Discussion

Results from these studies clearly showed that there was a strong association between the potato psyllid and ZC, both in the greenhouse and field experiments. Plants exposed to psyllids developed ZC symptoms in raw tubers and fried chips. In the greenhouse, 67.5% of the plants showed foliar symptoms, whereas 25.8% and 59.2% showed ZC symptoms in the raw tubers and fried chips, respectively (Table 1). In the Yakima field experiment (2006), 42.5% of potato plants showed foliar symptoms, 15% of raw tubers exhibited ZC symptoms, and 57% of tubers showed ZC symptoms in the fried chips (Table 2). At harvest, results from the Weslaco field experiment (2007) showed that potato plants exhibiting ZC symptoms in raw tubers averaged 79.2, 37.5, and 48.6% for uncaged plants, caged plants infected with Texas field collected psyllids, and caged plants infected with laboratory-reared psyllids, respectively (Table 3). Plants showing typical ZC symptoms in fried chips averaged 87.5, 52.8, and 63.9% for uncaged plants, caged plants with Texas field collected psyllids, and caged plants with laboratory-reared psyllids, respectively (Table 3). No single potato plant in the greenhouse rooms and field cages without psyllids (controls) exhibited ZC foliar symptoms or showed ZC symptoms in raw tubers or fried chips during the experiments, suggesting that the observed ZC symptoms were due to psyllids. None of the potato plants or tubers with psyllid damage or typical ZC symptoms collected from the greenhouse or field cages tested positive for phytoplasmas in nested PCR using non specific (universal) primer pairs P1/P7 and fU5/RU3, and fU5/BLTVA-int, the specific primer pair for the Columbia Basin potato purple top phytoplasma, commonly found in the Pacific Northwest of the United States and that causes plants symptoms resembling ZC (Crosslin et al. 2006; Munyaneza et al. 2006). This suggests that the observed ZC symptoms in the fried chips were due to psyllid damage or other pathogens than phytoplasmas.

Table 1. Potato plants, tubers, and chips exhibiting psyllid damage and zebra chip symptoms resulting from potato plants exposure to the potato pysllid under greenhouse conditions (Yakima, WA, 2006)[1]

Potato cultivar[2]	% Plants with psyllid damage symptoms	% Raw tubers with zebra chip symptoms	% Fried chips with zebra chip symptoms
Atlantic	90	36.7 ± 2.1a	70.0 ± 0.4a
FL 1879	70	33.3 ± 0.9a	63.3 ± 1.1a
FL 1833	60	20.0 ± 1.8a	46.7 ± 0.9a
FL 1867	50	13.3 ± 0.2a	56.8 ± 0.8a

[1]No plant or tuber zebra chip symptoms were observed in the potato psyllid-free greenhouse rooms
[2]Means followed by the same letter within columns are not significantly different (P> 0.05; LSD)

Table 2. Potato plants, tubers, and chips exhibiting psyllid damage and zebra chip symptoms resulting from potato plants exposure to the potato psyllid under field conditions (Yakima, WA, 2006)[1]

Potato cultivar[2]	% Plants with psyllid damage symptoms	% Raw tubers with zebra chip symptoms	% Fried chips with zebra chip symptoms
Atlantic	52.5 ± 3.2a	21.3 ± 1.2a	57.5 ± 1.8ab
FL 1879	45.0 ± 1.9a	17.5 ± 1.7ab	66.3 ± 0.9a
FL 1867	30.0 ± 2.2a	6.3 ± 0.5b	47.5 ± 1.3b

[1]No plant or tuber zebra chip symptoms were observed in the potato psyllid-free field cages
[2]Means followed by the same letter within columns are not significantly different (P> 0.05; LSD)

ZC is an important potato disease that is causing serious losses to the potato industry in North and Central America. A good understanding of this disease is essential to develop effective management strategies to reduce damages caused by this disease in affected areas. Results of the present studies indicate that ZC is strongly associated with the potato psyllid. However, mechanisms by which this insect induces ZC symptoms in potato are still not known. It is suspected that this insect injects unknown plant pathogens or toxins in potato plants when feeding. It is well documented that the potato psyllid induces psyllid yellows disease by injecting toxins in susceptible host plants (Wallis 1955). It is also known that other species of psyllids transmit plant pathogens, including phytoplasmas, bacteria, and viruses (Munyaneza et al. 2007a). During the present study, we could not determine if this insect was causing ZC by transmitting toxins or pathogens. Recent studies have shown that ZC is graft-transmissible for several generations (Crosslin, unpublished data). However, extensive testing of ZC symptomatic plants and tubers has so far failed to detect any known plant pathogen. On the other hand, it is not clear on how toxins can be graft-transmitted, especially for several generations. Further studies are underway to try to identify ZC causal agents.

Table 3. Potato plants with zebra chip symptoms in raw tubers and fried chips resulting from potato plants exposure to the potato psyllid under field conditions (Weslaco, TX, 2007); varieties were pooled.

Treatment	Percentage of plants with ZC symptoms in raw tubers (Mean ± SEM)	Percentage of plants with ZC symptoms in fried chips (Mean ± SEM)
Uncaged plants	79.2 ± 10.5c	87.5 ± 5.4c
Caged plants with Texas field collected psyllids	37.5 ± 8.8b	52.8 ± 5.6b
Caged plants with laboratory reared psyllids	48.6 ± 9.0b	63.9 ± 8.2b
Caged plants without psyllids	0.0 ± 0.0a	0.0 ± 0.0a

Means followed by the same letter within columns are not significantly different ($P > 0.05$; LSD).

In conclusion, results of the present studies showed that ZC was strongly associated with the potato psyllid. However, ZC causal agents are still unknown. Information from this study will help potato growers in ZC affected areas minimize damages caused by this disease by developing effective monitoring and management strategies targeted against this insect pest. However, to develop effective management strategies for control, it remains imperative that ZC causal agents be identified and mechanisms by which this potato psyllid induces ZC symptoms be further investigated.

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PHYTOPLASMA DISEASES OF POTATOES IN THE NORTHWEST UNITED STATES

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Approximately half of the potatoes grown in the United States are produced in the Pacific Northwest states of Washington, Idaho and Oregon. In the 1940's and 1950's in Oregon and Washington, a disease of potatoes was reported that produced symptoms similar to those of aster yellows. The disease was associated with the aster leafhopper, *Macrosteles fascifrons* (Raymer and Milbrath 1960; Hagel 1974). At that time there were no molecular tests available for positive identification of the causal agent as a phytoplasma. In the Columbia Basin of Washington and Oregon in 2002, symptoms of phytoplasma infection of potatoes were widespread and included purple discoloration of shoot tips, leafrolling, and aerial tubers. Nested PCR with "universal" primers P1/P7//fU5/rU3 detected a phytoplasma in most of the plants showing these "purple top" symptoms (Crosslin et al. 2005). The DNA sequence of the PCR products indicated that the causal agent was a phytoplasma in the clover proliferation group (16SrVI) that it is related to, or synonymous with, the beet leafhopper transmitted virescence agent (BLTVA; Golino et al. 1989). Similar results were obtained by other workers (Lee et al. 2004b) with symptomatic potato samples from Oregon and Washington. Subsequent PCRs using primers P1/P7/fU5/BLTVA-int specifically detected this phytoplasma (Crosslin et al. 2006). In our recent studies, the aster yellows phytoplasma (group 16SrI) has not been detected in potatoes grown in Washington or Oregon that show symptoms of purple top, although aster yellows has been detected in carrots and onions grown in the region. Beet leafhoppers (*Circulifer tenellus*) successfully transmitted BLTVA to numerous cultivars of potatoes and several species of weeds including *Senecio vulgaris*, *Capsella bursa-pastoris*, *Kochia scoparia*, and *Salsola kali* (Munyaneza et al. 2006). PCR tests of individual beet leafhoppers indicate that the incidence of infection with the phytoplasma is quite high and averages approximately 10-15% depending on collection site and date. Phytoplasma has been detected in over-wintered adult leafhoppers. Additional tests have shown that the phytoplasma is tuber-borne at a relatively high rate in many commercially grown cultivars and can lead to phytoplasma-infected daughter plants.

Another group 16SrVI phytoplasma has been found on potatoes in Alaska (Lee et al. 2004a) and causes witches'-broom symptoms that are quite distinct from those caused by BLTVA. The vector of this phytoplasma has not been determined. PCR results on several species of leafhoppers collected in Alaska indicate that other phytoplasmas, including aster yellows, are present in Alaska. BLTVA was reported in radish seed crops in Idaho (Shaw et al. 1990), but to our knowledge has not been positively identified in potatoes from that state.

Phytoplasmas have been reported on potatoes from around the world. Some of these reports include groups 16SrI and 16SrII reported on potatoes in Mexico (Leyva-Lopez et al 2002), group 16SrVI from Korea (Jung et al. 2003) and Canada (Khadhair et al. 1997), and more recent reports of groups 16SrI and 16SrXII (or related) on potatoes in the central United States (Secor et al. 2006; Lee et al. 2006) and Russia (Girsova et al. 2008). These reports and our on-going research indicate that diverse phytoplasmas infect potatoes around the world and pose an economic threat to this important food crop.

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Pathology

IDENTIFICATION OF SOIL PATHOGENS ASSOCIATED WITH SUPERFICIAL BLEMISHES ON POTATO TUBERS

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Introduction

Potato tubers are nowadays washed before selling: this process reveals diverse superficial blemishes leading to downgrade or reject up to 20% of the production although these visual defects do not impair the organoleptic quality of the tubers. These superficial blemishes include many different types of symptoms among which some are well known as caused by identified pathogens (Anonymous, 2001). This paper concentrates more specifically on netted scab-like or corky or superficial russeting symptoms excluding typical common scab lesions caused by *Streptomyces reticuliscabei* and *S. europaeiscabiei* (Bouchek-Mechiche *et al.*, 2000). Those symptoms will be referred as atypical symptoms and their aetiologies are still to be better assessed. From preliminary data (Campion *et al.*, 2003), star-shaped netted scab like lesions have been attributed to *Rhizoctonia solani* AG 2-1 and *R. solani* AG 5 but all Koch's postulates have not been fulfilled so far.

The aim of this work is i) to carry out diagnosis of pathogens associated with superficial blemishes, ii) to characterize the pathogenic agents associated with most frequently encountered tuber symptoms with unidentified causal agents (and vice versa); this involves classical and molecular identification of the micro-organisms isolated from the symptoms and Koch's postulates fulfilment, iii) to relate environmental factors and symptom occurrences with the ecological requirements of the pathogenic agents to suggest strategies to control their infectious activity. Only points i) and ii) will be presented hereby.

Materials and methods

1. Symptom classification

Potato tuber symptom collections were conducted at harvest in fall 2006 and 2007. Seed as well as ware potato fields were investigated across various French potato production areas (North, West and Center areas). Superficial symptoms of 204 samples were qualitatively described, digitally recorded and classified according to their morphological aspect. Typical and well identified samples, like netted and pitted common scab and tubers with black scurf (sclerotia of *Rhizoctonia solani*) were scored according the Seed Certification Chart (Anonymous, 1985)

2. Microbial isolation and identification

A small piece of tuber surface was taken out from the symptomatic region of each tuber with a press-knife. The explant was sanitized into a chlorine bleach bath and rinsed into three consecutive distilled water baths. It was aseptically air dried and laid on potato dextrose agar (PDA) or water agar in Petri dishes. Fungi that developed from the explant were isolated.

For *Streptomyces* spp. isolation, infected tubers were washed under running tap water for 5 min. One gram of diseased tissue was cut aseptically from the tubers, homogenized for a few minutes in a sterile mortar and serially 10 fold diluted to 10^{-6} in sterile water. One mL aliquots of the 10^{-5} and 10^{-6} dilutions were added to 100 mL of tyrosine–casein–nitrate agar medium (Waksman, 1961) maintained at 50°C and the inoculated medium was then poured into Petri dishes (15 mL per dish). The plates were incubated at 27°C for 10 days, and colonies of *Streptomyces* were transferred to PDA (Pridham *et al*., 1956–57). Colonies were purified at least twice by serial transfers on the same medium (Bouchek-Mechiche *et al*., 2000).

First, fungi were identified by microscopic observations. Then, they were grown in potato dextrose broth (PDB) and mycelia were filtered, lyophilised and ground. The DNA was extracted with the DNeasy plant mini kit (Qiagen) and the ribosomal internal transcribed spacer (ITS) region was amplified by polymerase chain reaction (PCR) using primers ITS1F (Gardes and Bruns, 1993) and ITS4 (White T.J. *et al.*, 1990). The PCR products were sequenced and sequence identities were determined using BLAST.

3. Pathogenicity test

The fulfilment of Koch's postulates was achieved under controlled conditions in a greenhouse for a representative group of 33 strains chosen among the fungi isolated in 2006. Sixteen (16) strains of *R. solani* AG3, 9 strains of *Fusarium* spp. and 8 others strains were tested. The test was carried out on high grade (SE) seed tubers of cv. Charlotte with three replicates for each fungal strain. Fungi were grown on millet seeds. This inoculum was mixed with sterilized soil in which a healthy tuber was planted. After 125 days, potatoes were harvested and symptoms on progeny tubers were scored according the French official seed certification chart (9 levels of severity).
Fifteen days before harvest, soil samples were taken out to check the presence of the inoculated fungi. About 20 g of soil were sampled 5 cm under the surface of the pot. Each sample was homogenised and some aggregates were spread on the surface of a Petri dish containing PDA. The developing fungi were observed under the microscope.

Results and discussion

1. Symptom classification

Field symptoms were classified according 9 different classes of superficial blemishes: typical netted and pitted scab lesions, sclerotia (black scurf), enlarged lenticels, netted-scab like (corky, russeting, elephant-hide, desquamation) lesions with regular or irregular contour lines: round, hallo and star-like, deformation and cracks (Figure 1). Most frequent classes, rated through percentage of occurrence within the sampled collections, are thoroughly described and the description for each classe will be submitted to a large panel of field experts for overall terminology associated with symptomatology.
Different potato cultivars were sampled: from the observed samples, an interaction "potato genotype" versus "microorganism" on the symptomatology cannot be excluded; a given microorganism (bacterium or fungus) could trigger different types of symptoms according to the potato genotype.

Figure 1: Netted scab like lesion (A) and enlarged lenticels (B).

2. Isolation and identification

A total of 329 fungi were isolated in 2006 and 2007 and 23 *Streptomyces* were isolated in 2007 (Figures 2 and 3).

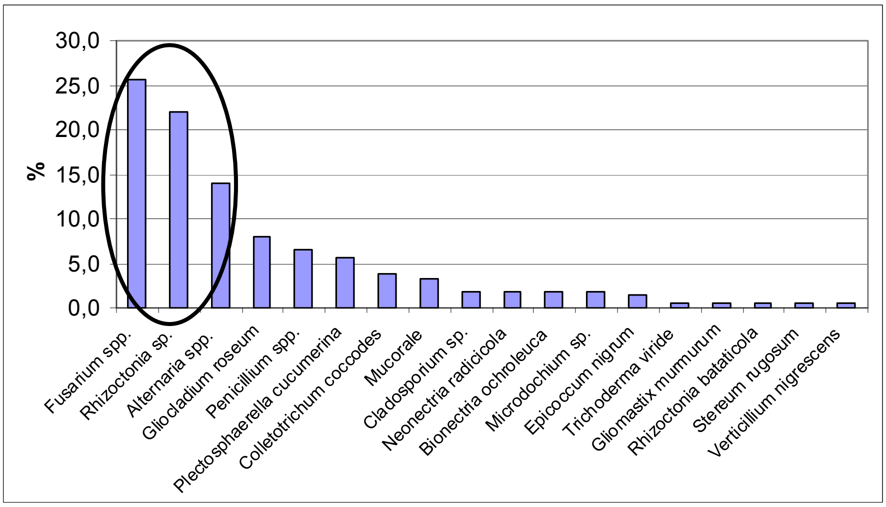

***Figure 2*: Fungal diversity obtained in 2006 (215 isolates, molecular identification).**

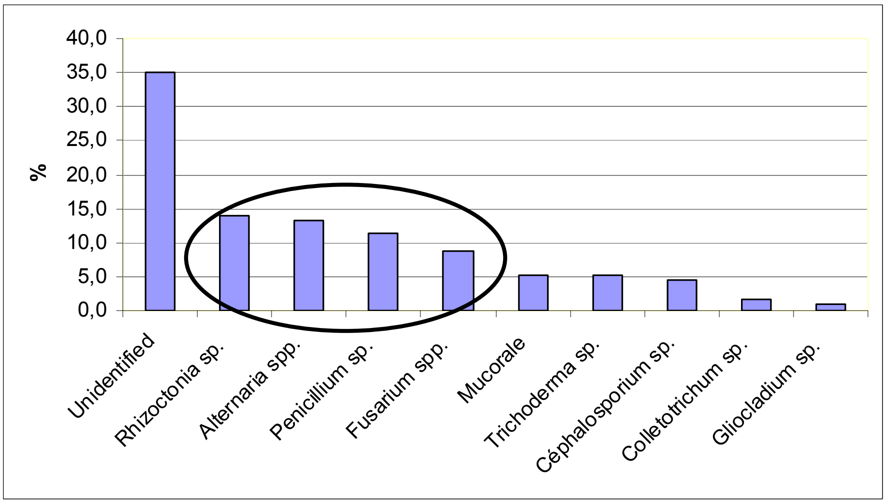

***Figure 3*: Fungal diversity obtained in 2007 (114 isolates, morphological identification).**

The genera *Fusarium*, *Rhizoctonia* and *Alternaria* were among the most frequent for the two years of isolation and especially *F. oxysporum* and *R. solani* AG 3. The ITS sequencing was not sufficient to identify the *Alternaria* strains at the species level. Other DNA regions will be sequenced to achieve this identification of *Alternaria* sp. Concerning the isolates obtained in 2007, thirty five percent of the fungi could not be identified morphologically. They were pooled in a single class named "unidentified fungi". The morphological observations enabled us to say that these fungi belong to several different genera which are not a part of the head genera. The molecular identification is in progress and will clarify their taxonomic position.

Fungal strains belonging to the same species were associated to various types of lesions and one type of lesion was caused by different species of fungi. Thus, there was no obvious relationship between taxa and types of symptoms.

To check whether the lesions are due to exactly the same fungus or to different fungi belonging to the same species, we will investigate the intraspecific genetic diversity of the fungal strains using amplified fragment length polymorphism (AFLP) method.

Streptomyces spp. were not isolated from star-shaped spots and netted scab like lesion but they were found in 63% of the cases of typical common and netted scab and "rhizoscab" symptoms. This suggests the implication of *Streptomyces* bacteria in the appearance of those types of symptoms. However *Streptomyces* spp. were not isolated from all the netted scab symptoms, which are known to be caused by *Streptomyces* bacteria. The isolation method needs to be adapted, maybe by shorter sanitization or lower dilutions unless other microorganisms are involved in the occurrence of netted scab symptoms.

3. Pathogenicity tests

At the end of the pathogenicity test, the presence of the inoculated fungi in the soil could not be confirmed for all the pots. In the inoculated soils, *Fusarium* isolates were found again in 50 % of the cases, *R. solani* was found only in 12.5 % and the other isolates were found in 62.5 % of the cases. On the other hand, *Fusarium* strains were isolated from harvested tuber in 19 % of the cases, *R. solani* in 76 % of the cases and the other strains were isolated in 26 % of the cases. These results show that the repartition of the fungi in the soil depends on the ecological fitness of the strain inoculated. For example *R. solani* seems to be attracted by the host plant. It is also possible that some inoculum did not survive into the soil. A more reliable sampling method could be used for the detection of the inoculum into the soil.

Thirty-one fungi out of the 33 strains were able to reproduce the atypical symptoms described earlier. However, only 10 out of 33 strains reproduced symptoms similar to the one from which they were isolated. One fungal strain could cause various types of symptoms and a given type of symptoms could be caused by fungi belonging to different taxa. The only clear relationship between a fungal species and a type of symptom was between *R. solani* and the occurrence of sclerotia. Apart from this obvious evidence, no particular relationship could be established.

The isolation and identification procedure combined with the bioassay confirmed the microbial nature of the superficial alterations of the tubers but also the diversity of the causal agents.

In order to relate the different symptoms with fungal species, potato varieties, geographic origins and types of soil, the global set of data will be analysed by correspondence analysis.

To test whether atypical symptoms are caused by single or combinations of strains, a new bioassay will be carried out, including bacterial and fungal strains. Additional strains of *R. solani*, *Fusarium* spp. and *Alternaria* spp. will be tested, both alone and in coinoculation tests. For this, a new bioassay in hydroponic conditions will be developed to visualize the symptoms on the tubers all along the culture.

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DRYCORE SYMPTOMS ON POTATOES REQUIRE BOTH RHIZOCTONIA SOLANI AG 3 INFECTIONS AND WOUNDS ON TUBERS

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Rhizoctonia solani Kühn (telemorph: *Thanatephorus cucumeris* (Frank) Donk) is a soil-borne plant pathogen affecting many agricultural crops worldwide (Ogoshi, 1987; Sneh et al., 1991). On potatoes (*Solanum tuberosum* L.) the disease causes delayed emergence, lesions on stems (stem canker) and stolons and sclerotial formation on tubers (black scurf). Infections on stolons may induce the development of malformed tubers and tubers with growth cracks (Jeger et al., 1996). Besides stem canker and black scurf, other symptoms have also been attributed to *R. solani*, most notably drycore. Drycore is characterized by restricted brown cavities up to several mm deep with a diameter of 3 to 6 mm (Schwinn, 1961; Grütte, 1940). To date, fourteen anastomosis groups (AG 1 to AG 13 and AG BI) and several sub-groups have been identified (Gonzalez et al., 2001, Carling et al., 2002). AG 3 has been known as the most host-specific type of *R. solani* causing disease on potatoes.

In a three year survey (2001 to 2003) on a total of 278 potato fields of different farming systems (organic, integrated and conventional) in Switzerland the occurrence of drycore was significantly more frequent and higher in all three years in the organic farming system, as compared to the other systems (Keiser, 2007). Drycore belongs to the most important quality deficiencies in organic potato production in Switzerland. The level of drycore damage was on the same level in all three years and was not reduced in the exceptionally dry and hot year 2003 (600 to 750 mm annual precipitation in all regions, compared to 1150 – 1450 mm and 1000 – 1300 mm in 2001 and 2002 respectively). These results are not consistent with Schwinn (1961) who postulated that drycore symptoms are caused if *R. solani* penetrates the tuber tissue via the lenticels, if these tend to form excrescences under wet conditions, especially in soils with higher clay content. Infections of *Rhizoctonia solani* on seed tubers or harvested tubers increased the risk for drycore, but potato lots with heavy black scurf infestation were often absolutely free of drycore. Thus the infection of a plant with *Rhizoctonia solani* seems to be a prerequisite but not the only condition for the formation of drycore symptoms on tubers. The infestation level of drycore in all farming systems was significantly influenced by wireworm damage, seed quality (sclerotia) and grass clover leys as a preceding crop. Important damage was found on all soil types independently from clay and organic matter content. Most isolates of *R. solani* from this survey were assigned to AG 3. Wireworm damage on potatoes was enhanced by grass clover leys in the crop rotation which might explain in part the higher occurrence of drycore in organic agriculture as it is much more common to grow potatoes after several years of grass clover leys.

Based on the results in the survey the hypothesis was put forward that injuries of wireworms on potato tubers facilitate the penetration of *R. solani* AG 3 into the tuber and favour the formation of drycore symptoms. This hypothesis was tested with open air experiments and pot experiments under controlled conditions. Isolates of *R. solani* originating from black scurf and drycore from harvested tubers were identified using pectic zymograms. ITS-rDNA primer specific amplification, ITS sequencing and ISSR-PCR fingerprinting was used to test if isolates of *R. solani* isolated from black scurf and drycore on harvested tubers shared identical fingerprint genotype to the isolates found on the seed tubers used in the field experiments and to the isolate used to inoculate the soil in the pot experiments. Drycore symptoms on harvested tubers were only observed in treatments where *R. solani* was present and potato tubers were injured either naturally by wireworm or artificially with needles.

Non-wounded tubers rarely had drycore symptoms, even when they were infested with black scurf. In the treatments without *R. solani* infection black scurf or drycore was not observed. All isolates of *R. solani* from black scurf or drycore symptoms belonged to AG 3. Furthermore, all isolates obtained from black scurf and drycore in the pot experiments presented an identical fingerprinting genotype as the isolate used to inoculate the sterilized soil.

The results of the experiments indicate that *Rhizoctonia solani* AG 3 is the causal agent of drycore and that wound on tubers caused by wireworms or other means favour the formation of drycore symptoms. In contrast to the generally accepted opinion that drycore is caused by *R. solani* if the lenticels tend to form excrescences under wet conditions, wounds by wireworms or other means seem to be of higher importance under the conditions of Swiss potato production. The higher occurrence of drycore in organic potato fields can be explained by the often higher infestation level of black scurf on organic seed lots (Keiser, 2007), the lack of effective treatments against *Rhizoctonia solani* and wireworm and the fact that potatoes are often grown after grass clover leys, which enhances the risk for wireworm damage on tubers. To reduce drycore damage the crop rotation has to be optimized, especially in the organic farming system. Our results suggest that a promising issue for future research might be to develop methods to produce seed free of black scurf in organic production and to find biological control methods against wireworms.

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INFECTION WITH *RHIZOCTONIA SOLANI* INDUCES DEFENCE GENES AND SYSTEMIC RESISTANCE IN POTATO SPROUTS

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Rhizoctonia solani is a common soilborne and seedborne fungal pathogen that attacks potato sprouts (*Solanum tuberosum*) prior to emergence, which is little studied. Initially, hyphae colonize densely a region just below the apex, which often kills the tip of the sprout (Richards 1921). The plant responds with initiation of new sprouts from the base of the damaged one. In these new sprouts severe symptoms are usually not observed and they emerge successfully (Sanford 1938). The aim of this study was to provide further insight into the mechanism by which the new sprouts recover from infection with *R. solani*.

Pathogen-free tubers of cv. Saturna were sprouted in cool and moist conditions in darkness to mimic conditions beneath soil. The basal part of the sprout was isolated from the apical part with a soft plastic collar and inoculated with highly virulent *R. solani* (anastomosis group AG-3) isolated from potato in Finland (Lehtonen et al. 2008). Induction of defence-related responses was monitored in the apical part using TIGR Potato cDNA Array [10K version 4; obtained from the National Scientific Foundation´s (NSF) Potato Functional Genomics Project] at 48 and 120 hours post-inoculation (hpi). The expression of 25 genes was also tested by quantitative realtime PCR. The top of sprouts was challenge-inoculated with *R. solani* 120 h after inoculation of the basal part. All treatments and handling of the plants were done in absence of photosynthetically active light.

Results revealed that potato sprouts developing in dark could respond to infection with *R. solani* by induction of strong systemic resistance that prevented infection of the apical part of the sprout following challenge inoculation. The systemic induction of resistance in the apical part of the sprout was manifested at the molecular level by differential expression of 122 and 779 genes at 48 and 120 hpi, respectively. Many induced genes were well-characterized defence-related genes that were upregulated as soon as 48 hpi when no apparent infection structures were yet detected at the base of the sprout. Hence, potato sprouts could recognize the invading hyphae of *R. solani* sensitively and quickly. To our knowledge, there is little previous knowledge of efficient pathogen defence not been in the subterranean parts of the potato plant, including sprouts prior to emergence.

The results of this study provide an explanation to a long-known phenomenon characteristic of the first phase of the disease caused by *R. solani* on potato. Initially, infection kills sprout tips before emergence, which is followed by initiation and compensatory growth of one or a few new sprouts from the base of the damaged sprout (Richards 1921). As noted already by Sanford (1938), "these secondary sprouts appear to possess a remarkable degree of resistance, notwithstanding the primary sprouts were very susceptible and severely attacked under apparently identical conditions". According to the data of this study, it is conceivable that initial infection with *R. solani* induces systemic resistance in sprouts also under the normal growth conditions in the field, which allows the new sprouts to resist secondary infection. Systemic induction of resistance in sprouts upon infection with virulent *R. solani* provides novel information about pathogen defence in potato before the plant emerges and becomes photosynthetically active. The results advance understanding of pathogen defence in subterranean parts of the plant, which is little studied.

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FUSARIUM GRAMINEARUM AS A CAUSE OF POTATO DRY ROT

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A survey of potato tubers with dry rot symptoms from commercial storages showed that 42% of the dry rot was caused by Fuarium graminearum (Fg). Isolates of Fg from potato, wheat, and sugarbeet all caused dry rot of potato tubers regardless of original host. An injury trial showed that Fg requires a substantially more invasive wound for infection compared to Fusarium sambucinum. A change in harvest practices may be responsible for the importance of dry rot caused by Fg.

BIOLOGIZED SYSTEM OF EARLY POYTATO PROTECTION FROM DISEASES

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In biologized system of potato protection from harmful objects on the base of long - term regulation of agrocoenosis components the main role is given to usage of a new class of fungicides and phytoregulators for emphasizing their natural protection plant reactions to unfavourable growth conditions and optimization of production processes. Biopreparations for plant immunization have ferment, lipid and carbohydrate nature. Such preparations are quite enough and they show different effectiveness on decrease of disease development and influence potato productivity in field experiments and potato growing practice in the Russian Federation regions.

Potato has the greatest reaction to immunizator cultivation in physiologically young tissues and organs, when plants are at 1…7 stage of organogenesis. Several method groups of acquired physiology – biochemical immunity stimulation are known. 1. Preinoculation of cormophyte plant by unpathogenes, weak, incompatible pathogene races, saprophytes, symbionts. 2. Application of chemical matters of different nature and physical factors. It is necessary to point out (Diyakov Yu.T., 2002) that alive inductors (bacteria, fungi) applications may make harm when compatible races used or their activity increased. So, it is more safe, profitable and effective to use cultural liquids, life activity products of thallome plants and chemical matters of different nature. Duration of physiology and biochemistry phytostability period after activators cultivavtion of disease resistant cormophyte plants may range from 3 to 8 weeks. The Sun radiant energy quanta as ecological factor are the basis of photosynthetic carbon dioxide fixation. Photosynthetic apparatus continuously reacts to changes of photons flood intensity and spectral composition of the Sun energy, integrity and effectiveness of both photosystem and Calvin cycle work. Even in average amounts of radiant energy intensity and carbon dioxide concentration in the earth air layer the speed of electron transportation is surplus in comparison with enzymathic reaction speed of photochemical carbon fixation. It may lead to restoration of electrical transport chain. Caratinoids which are contained in large amounts in biopreparations of Verva and Albit, play great role in relaxing energetic intensity in cells. As our research showed treatment of early potato plants by these biopreparations considerably increases chlorophyll and caratinoide content in plant leaves in all term consideration, which have intensive colour before harvesting. Application of disease resistant inductors of embrio plants is an effective method of phytosanitary agrolandscape optimization under biologized protection system in the integral early potato biotechnology.

In experiments carried out in 2005-2007 the experiment scheme included the following variants. Factor A. Early potato protection system from diseases: 0) wetting tubers and two vegetation sprayings by water – control (zero variant. 1) Tuber treatment by TMTD and two vegetation sprayings in budding and in 10 day by Acrobat MC . 2) Tuber treatment, in budding and in 10 day by Phytosporin – M. 3) Tuber treatment, in budding and in 10 day by Gumi 20 – M. 4) Tuber treatment, in budding and in 10 day by Gumi 20-M, + Phytosporin – M. 5) Tuber treatment, in budding and in 10 day by Agat – 25 K. 6) Tuber treatment, in budding and in 10 day by Albit. 7) Tuber treatment, in budding and in 10 day by Binoram. 8) Tuber treatment, in budding and in 10 day by Novosil. 9) Tuber treatment, in budding and in 10 day by Verva. 10) Tuber treatment by Maxim, in budding and in 10 day by Ridomil Gold MC. 11) Tuber treatment by Phenoram super, in budding and in 10 day by Tanos. Factor B. Fertilizer doze: 0) without fertilizers – control. 1) Rated doze for 20 t/ha of tubers. 2) Rated doze for 30 t/ha of tubers.

As in previous years resistant inductors application and rated dozes of fertilizers considerably raises field germination (up to 96-99,8%) and amount of shooting buds in eyes, especially in the variant with Binoram, comparing with control variant. The greatest indices of leaf mass, assimilation surface area and amount of stems both from one shrub and from seeding area unit, amount and tuber mass under shrub (especially during the blossom period + 20 days) were marked in Albit and Binoram variants when organic mineral fertilizers used (Table 1). Treatment with Binoram decreased these indices in comparison with control variants and application of Phytosporin – M changed these indices inconciderably.

Application of Albit in early potato with rated dozes of fertilizers raised early potato productivity in great level (Table 2). Addititon to control variants made 1,3 t/ha without fertilizers and accordingly 4,5 t/ha and 5,2 t/ha with rated dozes of mineral fertilizers "Kemira – universal".

Table 1 Maximum square of an early potato leaf of "Nevsky" variety depending on fungicide application. Experimental and training farm "Milovskoye", Federal State Educational Institution of Higher Professional Education Bashkir State Agrarian University, 2005-2007, thousand m²/ha

Preparation	Fertilizer dose		
	Control	Rated portion for 20tones per hectare	Rated portion for 30tones per hectare
Water treatment	31,5	42,5	45,0
TMTD + Acrobat MC	33,5	47,9	51,0
Phytosporin M	33,0	45,7	49,2
Gumi-20M	33,3	47,6	50,9
Agat 25K	33,5	47,5	50,5
Albit	33,5	48,0	51,0
Binoram	32,4	43,9	46,1
Biosil	32,4	44,3	46,9
Verva	33,6	48,2	49,7
Maxim+Ridomil Gold MC	33,5	46,7	49,5
Gumi-20M + Phytosporin M	33,3	47,6	50,7
Phenoram super + Tanos	33,1	47,8	50,8

Table 2 Tuber yield of early potato of "Nevsky" variety depending on fungicide application. Experimental and training farm "Milovskoye", Federal State Educational Institution of Higher Professional Education Bashkir State Agrarian University, 2005-2007, tones per hectare

Fungicide	Fertilizer dose		
	Control	Rated portion for 20tones per hectare	Rated portion for 30tones per hectare
Water treatment	19,1	26,6	29,0
TMTD + Acrobat MC	20,2	30,2	33,0
Phytosporin -M	19,9	28,7	31,4
Gumi-20M	20,2	30,0	33,0
Agat 25K	20,3	29,9	32,7
Albit	20,4	31,2	34,2
Binoram	19,5	27,8	30,1
Biosil	19,6	27,6	30,2
Verva	20,5	30,6	33,4
Maxim+Ridomil Gold MC	20,5	29,4	32,2
Gumi-20M + Phytosporin -M	20,6	30,2	33,2
Phenoram super+Tanos	20,3	30,1	32,9
LSD_{05}	0,8		

At the same time improvement tendency of tuber yield quality when using protection reaction activators of different nature while accounting of fertilizer rated dozes was marked. Our research showed that bioregulators and different dozes of fertilizers influence the early potato yield quality (Table 3). Application of Albit raised starch content to 0,2%, while control variants have 17,8%.

Mineral fertilizers influenced in decreasing starch content to 0.15-0,25%. Application of Albit raised dry matter content to 0,3% , while control variant have 25,6%. Mineral fertilizers influences in decreasing starch content to 0,29 - 0,86%. Application of Albit raised vitamin C content to 1mg (control -21,4%). Mineral fertilizers influenced in decreasing vitamin C content to 0,31-0,32%. Tuber output in experiments made 93,0-93,8%. Application of Albit raised tuber output to 0,9%.

During the research period biological effectiveness of Albit application to late blight, while determining diseases during vegetative period, made 52,5 – 63,7% and as for tubers it was higher and made 99,8%. In macrosporioze it was not so high because of inconsiderable spreading of this disease in that year experiment and made about 24,5 to 31,2%. Biological effectiveness of tuber scub while harvesting was on a high level for such difficult for curing infection (36-41%). So, we consider that stable high effectiveness of Albit to all fungi diseases of early potato in the Republic of Bashkortostan was found (Tables 4 and 5).

Table 3 Tuber quality of early potato of "Nevsky" variety in experiments of 2005-2007.

Growth regulators	Dry matter, %			Starch content, %			Nitrate content, mg/kg			Ascorbic acid mg, %			Output ,%		
	Factor B			Factor B			Factor B			Factor B			Factor B		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Water	25,7	25,3	25,1	18,1	17,8	17,7	64	73	76	22,6	22,2	22,1	91,2	92,6	93,1
TMTD + Acrobat MC	25,8	25,5	25,3	18,1	17,9	17,8	64	72	76	22,6	22,3	22,1	91,4	92,7	93,2
Phytosporin - M	25,8	25,5	25,3	18,2	18,0	17,8	64	72	75	22,7	22,3	22,2	91,4	92,9	93,4
Gumi – 20M	25,9	25,6	25,4	18,3	18,0	17,9	64	72	74	22,7	22,4	22,2	91,6	93,0	93,5
Agat 25K	25,9	25,6	25,5	18,3	18,0	17,9	64	72	75	22,7	22,4	22,2	91,5	93,0	93,6
Albit	25,9	25,6	25,5	18,3	18,0	17,9	64	72	75	22,7	22,4	22,2	91,6	93,0	93,6
Binoram	25,8	25,4	25,3	18,1	17,8	17,8	64	72	76	22,6	22,3	22,0	91,3	92,7	93,0
Biosil	25,9	25,5	25,4	18,2	17,9	17,9	64	72	75	22,7	22,4	22,2	91,4	92,9	93,3
Verva	26,0	25,6	25,5	18,4	18,0	18,0	63	71	74	22,8	22,4	22,3	91,8	93,0	93,6
Maxim+Ridomil Gold MC	25,8	25,5	25,3	18,1	17,8	17,8	64	72	75	22,6	22,3	22,1	91,3	92,7	93,2
Gumiи-20M+PhytosporinM	26,3	26,0	25,6	18,5	18,1	18,0	63	71	74	22,8	22,4	22,3	91,8	93,1	93,7
Tanos	25,7	25,3	25,1	18,0	17,8	17,7	64	73	76	22,5	22,2	22,1	91,1	91,6	93,0

Table 4 Late blight in early potato experiment of "Nevsky" variety depending on biopreparation application

on leaves. Experimental and training farm "Milovskoye", Federal State Educational Institution of Higher Professional Education Bashkir State Agrarian university, 2005-2007, 08.08.

Препарат	Development degree %			Distribution %		
	Fertilizer doze			Fertilizer doze		
	control	Rated portion for 20tones per hectare	Rated portion for 30tones per hectare	Control	Rated portion for 20tones per hectare	Rated portion for 30tones per hectare
Water treatment	12,3	10,7	8,3	27,8	20,3	15,6
ТМТД+Acrobat МЦ	8,7	7,2	7,2	10,9	9,4	8,5
Phytosporin -M	9,9	7,7	7,5	11,3	10,2	10,1
Gumi-20M	9,2	7,3	7,1	11,0	9,7	9,2
Agat 25К	9,9	8,2	7,7	12,2	10,8	10,5
Albit	8,9	6,2	6,6	10,1	8,6	7,4
Binoram	12,7	9,9	8,1	16,2	14,3	13,7
Biosil	12,4	9,9	8,1	15,6	14,0	13,2
Verva	8,8	7,0	6,9	10,7	9,4	8,6
Maxim+Ridomil Gold МЦ	8,6	7,0	6,9	10,7	9,4	8,5
Gumi-20M + Phytosporin -M	8,8	7,2	6,9	10,9	9,5	8,6
Phenoram super+Tanos	8,5	7,0	6,8	9,2	7,6	7,2

Table 5 Biological effectiveness of fungicides against the late blight in early potato experiment of "Nevsky" variety on tubers at the harvest day. Experimental and training farm "Milovskoye", Federal State Educational Institution of Higher Professional Education Bashkir State Agrarian university, 2005-2007, 08.08.

Preparation	Biological effectiveness %		
	Fertilizer doze		
	Control	Rated portion for 20tones per hectare	Rated portion for 30tones per hectare
Water treatment	-	-	-
ТМТД+Acrobat MC	68,7	67,2	67,2
Phytosporin -M	0,9	0,7	0,5
Gumi-20M	49,2	47,3	47,1
Agat 25К	10,9	9,2	8,7
Albit	78,9	89,2	99,8
Binoram	4,7	3,9	3,1
Biosil	14,4	19,9	18,1
Verva	78,8	87,0	96,9
Maxim+Ridomil Gold MC	88,6	97,0	97,9
Gumi-20M + Phytosporin -M	48,8	47,2	46,9
Phenoram super+Tanos	88,5	97,0	96,8

REVUS - SETTING NEW STANDARDS IN THE POTATO CHAIN

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Potatoes do today and will play in future a key role in the diet of the Europeans. The trend is to eat fewer potatoes; however we eat more processed potatoes. In Europe we produce 40 % of the global potato production and we assume 50 % of it is processed. Potatoes produce more nutritious food more quickly than most other crops and up to 85 % of the plant is edible and potatoes produce more starch per hectare than any other crops.

The consumer requirements in Europe are shifting toward healthy food, convenience, tasty, look and feel, responsible production, care for the environment and low level of contaminants like crop protection residues. Potatoes, either fresh or processed have to comply with these requirements to keep their position in the value chain.

This asks from the potato grower a professional management of the crop. Crop protection plays a key role to produce high yields and consistent quality. Without crop protection use the yield and quality loss in potatoes will be about 75 %.

Syngenta develops crop protection products and crop protection services to support growers in meeting these challenges. One of the latest innovations is the blight fungicide Revus, based on Mandipropamid, with an excellent profile that meets today's requirements on worker -safety, environmental - and consumer safety.

DICKEYA SP. (SYN. *ERWINIA CHRYSANTHEMI*) SLOW WILT IN ISRAEL IN POTATO CROPS GROWN FROM IMPORTED SEED TUBERS

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Suspected *Dickeya* sp. isolates were obtained from potato plants and tubers collected from commercial plots. The disease was observed on crops of various cultivars grown from seed tubers imported from The Netherlands during the spring seasons of 2004-2007, with disease incidence of 2-30% (10% in average). In addition to typical wilting symptoms on the foliage, in cases of severe infection, progeny tubers were rotten in the soil. Six isolates were characterized by biochemical, serological and PCR-amplification. All tests verified the isolates as *Dickeya* sp. The six potato strains from Israel were biochemically identical to strains recently isolated from Dutch seed potatoes, and identified as *D. dadanti* by Rep-PCR analysis. One of the isolates was used for pathogenicity assays on potato cvs. Nicola and Mondial. Symptoms appeared 2 to 3 days after stem inoculation, and 7 to 10 days after soil inoculation. The control plants treated with water, or plants inoculated with *Pectobacterium carotovorum*, did not develop any symptoms with either method of inoculation. The identity of *Dickeya* sp. and *P. carotovorum* re-isolated from inoculated plants was confirmed by PCR and ELISA.

A protocol for detecting *Dickeya* latent infections in seed tubers has been developed using ELISA and PCR. Its reliability was tested by follow up the disease incidence in the field.

ASPECTS IN PATHOGENICITY OF *COLLETOTRICHUM COCCODES*, THE CAUSAL AGENT OF BLACK DOT IN POTATO AND ANTHRACNOSE IN TOMATO

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Colletotrichum coccodes (Wallr.) S. J. Hughes, the causal agent of black dot in potato and anthracnose in tomato, is a major pathogen on these hosts worldwide. The objectives of the present study were to understand the disease mechanism and to isolate pathogenicity-related genes. Identification of reduced- or impaired-virulence mutants depends on a rapid, reliable, large-scale screening method. The current bioassays for assessing *C. coccodes* aggressiveness use potato plants or tissue culture plantlets. Such bioassays are labor-intensive and time-consuming. A bioassay using ripe tomato fruit puncture was found to be unsuitable for classifying the aggressiveness of *C. coccodes* isolates. We have developed an alternative bioassay, in which the conidial suspension is injected into the stem scar of a mature green tomato fruits. Seven days later, the fruit is sliced and the lesion behind the stem scar is measured. This bioassay has demonstrated its reliability in determining isolate aggressiveness, it is faster and less costly than currently used bioassays and it can easily be applied to large-scale screening. The assessed aggressiveness of different *C. coccodes* isolates using tomato and potato bioassays was highly correlated. This newly developed bioassay enabled us to differentiate among wild-type isolates, based on aggressiveness. The most aggressive isolates secreted pectate-lyase (PL) 6 hours post induction at pH 6.0, while less aggressive isolates secreted the enzyme 12 hours post-induction. (conducted with anti-PL from *C. gloeosporioides* kindly provided by Prof. Prusky). The *C. coccodes* PL sequence was cloned by PCR using degenerate primers to the conserved areas of the enzyme and the whole sequence was isolated through DNA walking. A recombinant DNA cassette containing partial PL sequence and hygromycine-resistance gene was used to transform germinating conidia of an aggressive isolate. Transformants were selected by hygromycine resistance. Two hygromycine-resistant lines were found to undergo gene disruption via homologous recombination. The gene disruption decreased pathogenicity to tomato fruit by 20%.

It was demonstrated that *C. coccodes* may act as a seed-borne pathogen in tomato. Seeds collected from inoculated fruit (14 days post inoculation), appeared necrotic but no sclerotia were visible under a light microscope. Nevertheless, when these seeds were placed on selective medium, typical colonies of *C. coccodes* developed around several seeds, and typical sclerotia including setae were observed on the seed coat. Seeds that were heavily covered with fungal structures failed to germinate; others that were less colonized deteriorated soon after germination, and some were affected at later seedling stages. Treatment with Fludioxonil reduced the incidence of infected seeds but did not abolish it completely.

Sub populations of *C. coccodes* from Europe, Israel, North America, Australia and South Africa were characterized by vegetative compatibility groups (VCG) analysis and pathogenicty. Eight sub-populations were detected in the isolates originated from Europe-Israel, seven in North-American isolates and four in Australian isolates. Isolates from South-Africa could not be characterized or assigned to VCGs because nit mutants were not generated. Limited interactions between sub-populations were observed. All isolates were pathogenic to potato.

RHIZOCTONIA SOLANI SOIL INFESTATION IN SWEDEN AND BIOFUMIGATION STUDIES *IN VITRO*.

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Introduction

Attack of potatoes by *Rhizoctonia solani* has been a serious problem in Sweden for a long time. Earlier work in the country with this disease was focussed on chemical and alternative treatments of seed tubers. Lately, the possibilities to use various Brassica crops to reduce both nematode and fungal soil infestations have attained increasing interest. Plants belonging to Brassicaceae contain sulphurous compounds, glucosinolates, which through hydrolysis are converted to volatile isothiocyanates, a process referred to as "biofumigation" (Sarwar et al., 1998). The aims of the work presented here were i) to investigate the occurrence of soil infestation under various crop rotations and soil types in different regions of Sweden, and ii) to study the influence of biofumigation on *R. solani* and possible side-effects on potatoes. Both studies are financially supported by the Swedish Farmers Association for Agricultural Research, SLF, and the second one is made in collaboration with Ann-Charlotte Wallenhammar, Rural Economy and Agriculture Society, Örebro.

Materials and methods

<u>Study I, investigation of *R. solani* soil infestation</u>

With the help of local advisers, soil samples have been collected from fields with a history of R. solani attacks of potatoes. Samples were taken from three spots of 25 m2 in each field and the positions of the spots were registered on maps or by GPS, thus making repeated sampling from the same spot possible. Sampling was done in the autumn after a potato crop, year without potatoes = 0, in the following spring, year without potatoes = ½, and at increasing number of years with other crops The rotations, soil type, pH etc. were well documented for each field. The soil infestation has been analysed by using minitubers as a catch crop. After thorough mixing of the soil it has been distributed in pots wherein the tubers have been planted. After around two months in the dark at +10°C and watering when needed, the occurrence of stem canker on underground parts and sometimes black scurf on the seed tuber has been registered. The cause of injury has been validated by observations under magnification where the mycelium of *R. solani* is visible and by plating small pieces of canker tissue on artificial media. Parts from the same soil samples have also been subjected to another catch method using *Quinoa* seeds as baits (ref) and their DNA is now being analysed by real- time-PCR at SCRI (Alison Lees). Also, direct analysis of sub-samples of dried soil will be performed. The results from these three methods will be compared in the near future.

<u>Study II, influence of biofumigation on radial growth of *R. solani*</u>

Macerated plant material from *Brassica juncea* v. *crispifolia* (Curled mustard), *Brassica juncea* (Indian mustard) and two cultivars, A, with normal glycosinolate levels, and B, with elevated levels according to the supplier) of *Sinapsis alba* (White mustard) and *Raphanus sativus* (Oil radish), except root tissue, was used in laboratory studies to investigate their ability to prevent radial growth of *R. solani*. This is a first step to evaluate various species and cultivars for their efficiency as biofumigation crops to control soil infestation.

Plants were cultivated in fields outside Umeå in northern Sweden in the summers of 2006 and 2007. They were harvested when fully blooming. In 2006 the plant material was frozen and in 2007 it was dried at 40°C and stored dry at room temperature until needed. When used in experiments, the frozen plant material was macerated using a household grinder and the dry material was ground in an experimental mill (Kamas industri AB, Malmö) and sieved at 1 mm Ø.

Glass desiccators (5.6 – 5.8l) or plastic boxes (3.25 l) were used in the studies. Various amounts of plant material were placed on the bottom of these treatment chambers and tap water was added to the dry ground material so that it became wetted through. Controls contained water only. In 2006, 50 g FW l^{-1} was used in experiments including two replicates and the experiments were repeated three times. In 2007 lower dosages were used including amounts of 0.065 – 10.0 g DW l^{-1}. In 2006 six inoculated open Petri dishes on a small frame and ten minitubers of cv. Kenebec were added and the treatment chambers were then quickly closed with lids. The containers were incubated in the dark at +10 °C for three (2006) or two (2007) weeks. The dishes were then removed and closed with lids and kept for another three weeks at room temperature. The radial growth was recorded once a week, starting at removal from the treatment chambers. The no. of spouts of the minitubers and their weight were recorded after storing them dark at room temperature for six weeks after removal from the chambers.

Results

Study I

Using minitubers as baits, *R. solani* soil infestation was detected most frequently in soil samples collected from fields where potatoes were grown the previous year. The proportion of positive samples progressively declined with increasing numbers of years without potatoes (Figure 1). No evident pattern regarding influence of specific crops in the rotations on the decline of infestation has been found. However, in the northern part of the country, there were very few positive samples compared with the southern part at a similar time span to potato crops.

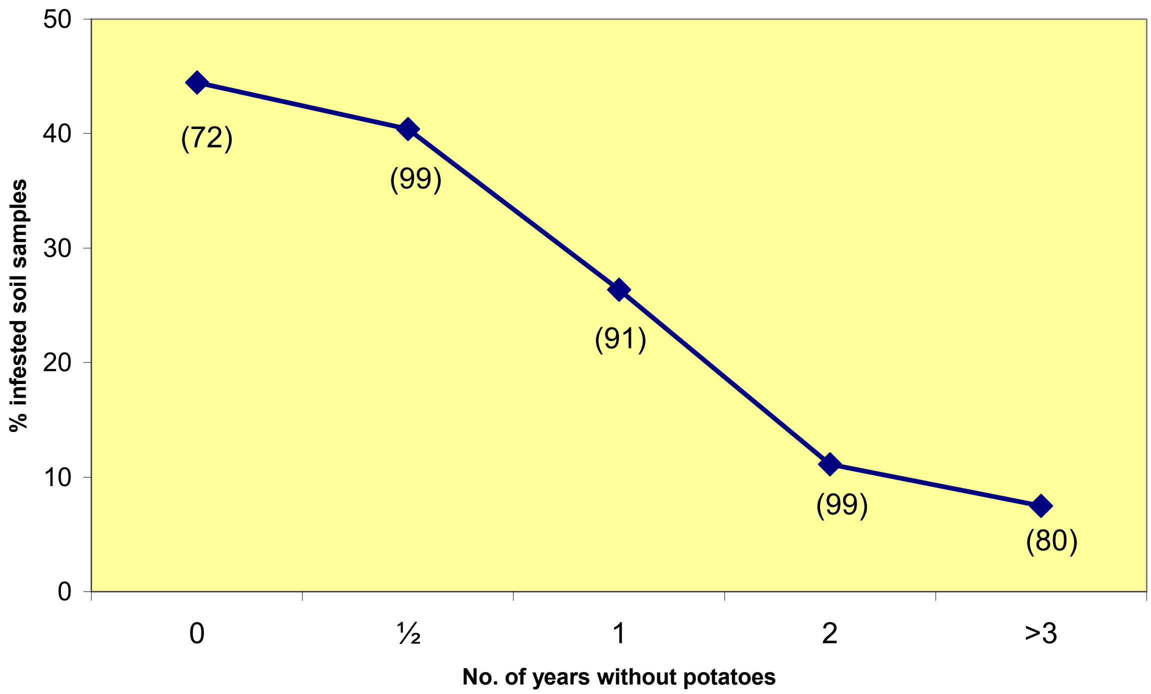

Figure 1. Percent soil samples found infested by Rhizoctonia solani in Sweden at increasing numbers of years without potatoes when using minitubers of potato as baits. The number of soil samples analysed at each point is given within parentheses.

Study II

Results from two similar experiments with *R. solani in vitro*, and from one experiment including also minitubers are presented in Figure 2. The two *Brassica juncea* species completely killed the *R. solani* inoculum and there was not any outgrowth of mycelium even three weeks after removal from the treatment chambers. The other species included as biofumigation material did not kill the fungus completely, leading to full mycelial cover in the Petri dishes after one week. The two *Brassica juncea* species also most effectively prevented sprouting of the minitubers. In the study presented, no difference of the actions between these two species was detected. However, at lower dosages used in later experiments the var. *crispifolia* was found to have stronger fungicidal action, data not presented.

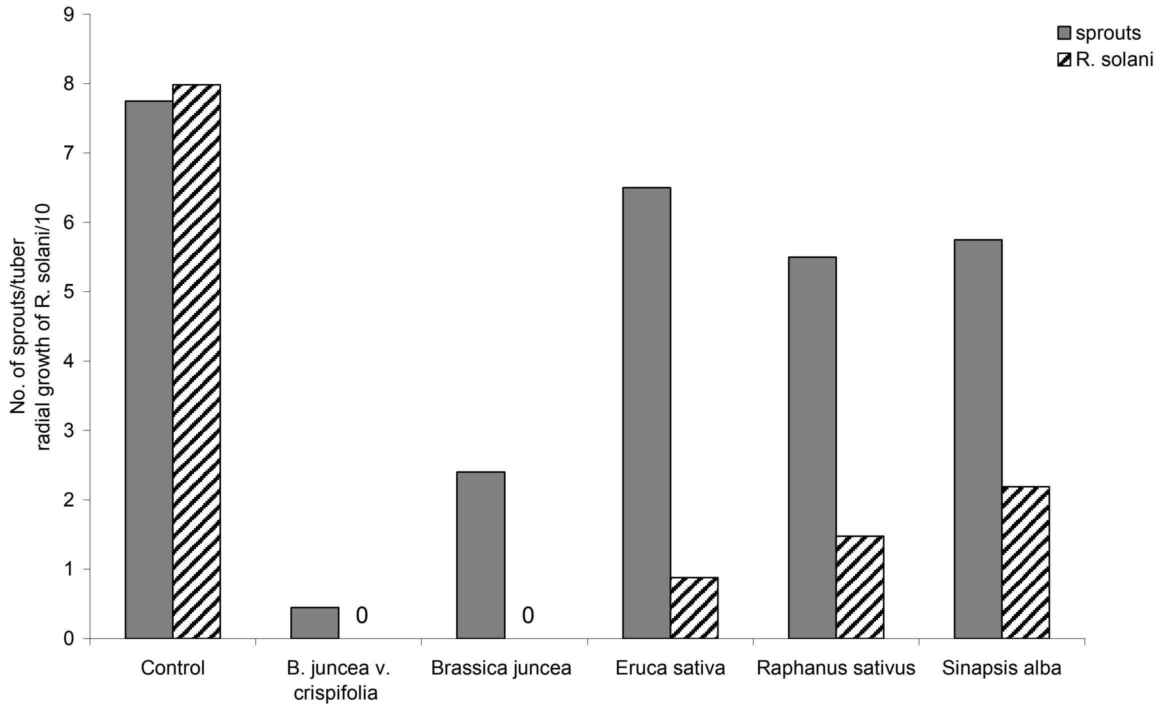

Figure 2. Radial growth of R. solani and no. of sprouts per tuber after treatments with volatiles of macerated plant material, as indicated, at 50g FW l^{-1} during three weeks. R. solani data refer to registrations of radial growth at removal from treatment chambers and are means of two similar experiments, tuber data are means of one experiment.

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DETECTION OF SYMPTOMLESS INFECTIONS OF *CLAVIBACTER MICHIGANENSIS* SSP. *SEPEDONICUS* USING A PCR BASED ON A VIRULENCE GENE

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Introduction

Bacterial ring rot, caused by the gram positive bacterium *Clavibacter michiganensis* ssp. *sepedonicus* (*Cms*) continues to remain a threat to the potato industry in North America. Although the disease occurs sporadically, serious outbreaks of bacterial ring rot have occurred in a number of potato producing areas over the past six years in the USA or Canada. Although bacterial ring rot has remained a 'zero tolerance' disease in the production of certified seed, this alone has been insufficient to effectively manage the disease since ring rot infections can remain symptomless, or latent, for some period of time. For this reason, post-harvest testing has been implemented in a number of areas in an effort to reduce the risk of seed borne infections of *Cms*.

A number of tests have been developed to detect symptomless infections of *Cms* in seed tubers. The first tests developed, and still in use today, were serologically based using *Cms*-specific monoclonal antibodies utilized in immunofluorescence (IFAS) (De Boer and Wieczorek, 1984) and enzyme-linked immunosorbent assay (ELISA) (De Boer, et al., 1988). ELISA has been demonstrated to be ineffective in the detection of nonmucoid or nonfluidal strains of *Cms*, however (Baer and Gudmestad, 1993). More recently, polymerase chain reaction (PCR) has been used and a wide array of primers have been developed to specifically detect *Cms* (Lee, et al., 1997; Li and De Boer, 1995; Mills, et al., 1997; Pastrik, 2000). The three *Cms*-specific primer sets developed by Mills, et al. (1997), Cms50, Cms72 and Cms85, are perhaps the most commonly used. However, of these three primer sets, Cms85 has been demonstrated to be less reliable and sensitive and is used less frequently than the other two primer sets (Baer et al., 2001). The objective of the studies reported here were to develop a PCR primer based on the cellulase virulence gene of *Cms* (Laine, et al., 2000; Nissinen, et al., 2001) for use in a real-time PCR format.

Materials and Methods

Freshly cut seed pieces from certified seed-tubers cv. Russet Burbank were vacuum infiltrated with ¼ strength nutrient broth containing either mucoid or nonmucoid *Cms* suspension of 10^8 or 10^4 cells / ml. Twenty-five seed pieces were planted in a 10m rows and harvested 127 days after planting. Approximately 20-30 asymptomatic tubers were selected from each treatment for use in evaluating existing and experimental diagnostic assays.

All detection assays were performed on stem end segments of tubers processed as previously described (Dineson and De Boer, 1995). Serological assays were performed also as previously described (Baer and Gudmestad, 1993). To mimic as closely as possible standard protocols followed by certification agencies in which 100 tuber core lots are processed, a single core from each infected tuber was blended with 99 *Cms*-free cores to determine the sensitivity and specificity of the CelA PCR assay compared to established methods of ELISA, IFAS and PCR with Cms50/72 primers. Presumptively healthy tubers for blending were selected from certified seed cv. Russet Burbank, processed as described above and confirmed by real-time PCR to be not infected with *Cms*. Each assay was performed at various levels of *Cms*-infected to healthy tubers such as 1 positive tuber per 100 tubers, 1 positive tuber per 200 total tubers, and 1 positive tuber per 400 total tubers. The soak liquid from *Cms*-free cores was used to make the dilutions such as 1:100 to 1:200 and 1:400 as each infected core could only be soaked once.

In addition to controlled experiments using simulated *Cms* contamination rates described above, certified seed lots known to be exposed to bacterial ring rot infections were also evaluated with all serological and PCR-based detection methods to compare detection efficiency. Some of these lots were tested using fresh tuber material processed as previously described (Dineson and De Boer, 1995), however, CelA was used to test frozen archived samples (-80C) after it was discovered that the seed lot was infected with bacterial ring rot. These naturally infected seed lots were tested using 4000 tubers/seed lot and all detection methods were applied to tuber cores processed into 200 tuber sub-lots. Symptoms of bacterial ring rot were quantified after these seed lots were determined to be infected with ring rot by one of the authors (Gudmestad) and the percentage of the disease was determined by scoring a minimum of 1000 plants per field.

***Cms* real-time PCR**

The standard set of primers used to detect *Cms* is based on the Cms50 and Cms72 fragments (Genbank Accession AY001266 and AY001267, respectively) determined by subtraction hybridization of *Cms* genome (Mills et al., 1997). This set amplifies single copy *Cms* specific DNA fragments of 192 bp and 213 bp respectively.

The flourogenic probe specific for Cms50 was labeled with reporter dye 6-carboxy-flourescein (FAM) at 5' end and the 3' end was modified with the quencher dye BH1. Similarly the probe specific for Cms72a was labeled with reporter dye Cy5 at 5' end and the 3' end was modified with the quencher dye BH2 (Sigma Genosys).

Cms50 probe: 5' [DFAM] TGAAGATGCGACATGGCTCCTCGGT [DBH1]3'
Cms72a probe: 5' [DCY5] GATCGTGAATCCGAGACACGGTGACC [DBH2]3'

Real-time (TaqMan) PCR was performed in 0.2ml optical tube strips (Stratagene) using Stratagene Mx3005P QPCR system. Real-time PCR involved addition of 4µl soak sample (diluted 1:20 with PCR grade water, Ambion) to an optimized mix consisting of 1X FastStart Universal Probe Master, Rox (Roche), both forward and reverse Cms50 primers at a concentration of 0.4µM, Cms72a primers at 1µM and the probes at 0.4µM and 0.6µM respectively. PCR grade water was added to a final volume of 25µl. A two step cycling protocol was used in the real-time PCR. An initial incubation of the mix at 95^0C for 10 minutes followed by 40 cycles at 95^0C for 30 sec, 60^0C for 45seconds and 72^0C for 30 sec.

The second primer, CelA-F and CelA-R were designed based on cellulase (CelA) gene encoded by the native plasmid pCS1 (Mogen et al., 1988) of *Cms* (Genbank Accession AY007311). The primers amplify a 150 bp region in the cellulase CelA gene. Sequences of the primers are described below.

CelA-F: 5'-TCTCTCAGTCATTGTAAGATGAT-3'
CelA-R: 5'-ATTCGACCGCTCTCAAA-3'

The flourogenic probe specific for CelA was labeled with reporter dye Hex at 5' end and the 3' end was modified with the quencher dye BH2.

CelA probe: 5' [DHEX] TTCGGGCTTCAGGAGTGCGTGT [DBH2]3'

Real-time PCR involved addition of 2µl soak sample (diluted 1:20 with PCR grade water, Ambion) to an optimized mix consisting of 0.8X FastStart Universal Probe Master, Rox (Roche), both forward and reverse CelA primers at a concentration of 0.75µM and the probe at a concentration of 0.16µM. PCR grade water was added to a final volume of 25µl. The real-time PCR protocol was identical as previously described for the Cms50 and Cms72 primer sets.

Specificity of the CelA primer was determined by testing in vitro *Cms* bacteria from our extensive culture collection in addition to other gram positive plant pathogenic bacterial species and bacterial pathogens of potato.

Results

The CelA primers detected all strains of *Cms* in our culture collection (>200) and did not detect other bacterial isolates. The other isolates used from our collection included *Clavibacter rathayi*, *Clavibacter tritici*, *Clavibacter xyli* subsp *cynodontis*, *Corynebacterium matruchotti*, *Curtobacterium flaccufaciens*, *Erwinia cartovora* subsp *atroseptica*, *Rhodococcus fascians* and *Streptomyces scabies*.

The CelA PCR primers also did not detect other subspecies of *Clavibacter michiganensis* such as *C.m.* ssp. *insidiosus*, *C.m.* ssp *michiganesis, C.m.* ssp *nebrakensis* and *Cm.* ssp. *tessellarius.*

The CelA PCR primers were more sensitive than other detection methods in detecting *Cms* infected cores that were blended with healthy tuber cores in simulation experiments (Table 1). The CelA primer was capable of detecting 2 *Cms*-infected cores blended with 198 healthy cores while the limit of detection in these experiments for IFAS and Cms50/72 primers were 10 infected cores in 190 healthy (Table 1). The CelA primer detected nonmucoid and mucoid strains of *Cms* with equivalent sensitivity.

Table 1. Sensitivity of three detection methods for Clavibacter michiganensis ssp. sepedonicus.

Infected/healthy cores	IFAS	Cms50/72	CelA
10/190	+	+	+
5/195	-	-	+
2/198	-	-	+
1/199	-	-	-

In naturally infected seed lots the CelA PCR primer was also more sensitive in detecting symptomless infections of *Cms* in seed tubers prior to planting (Tables 2 & 3). Among seed lots in which fresh tubers were tested, ELISA was completely ineffective in detecting *Cms* despite ring rot disease levels of >4% (Table 2). IFAS detected symptomless *Cms* infections in 2 of 3 seed lots while Cms50/72 PCR tests detected the ring rot bacterium in all three seed lots (Table 2). However, the number of sublots found *Cms*-infected using CelA PCR was proportionately higher than any other tests performed. Given the extremely high levels of ring rot observed in the field, with one seed lot have infection levels approaching 20%, it appears that CelA was more efficient in detecting symptomless *Cms* infections.

Table 2. Sensitivity of four detection methods for Clavibacter michiganensis ssp. sepedonicus among naturally infected seed of 4000 tubers in 200 tuber sub-lots.

Field Number	ELISA	IFAS	Cms50/72	CelA	BRR (%)
Field 1	0/20	2/20	6/20	13/20	4-6
Field 2	0/20	0/20	8/20	14/20	17-20
Field 3	0/20	1/20	1/20	5/20	6-9

Similar results were obtained from archived frozen samples that were tested after seed lots had been planted in the field and found to have ring rot infection levels of <1% (Table 3). ELISA, IFAS and Cms50/72 PCR tests were performed on these seed lots before they were planted and were negative (Table 3). Since these post-harvest lab tests were negative the commercial grower planted these seed tubers. CelA PCR was performed on the archived sample after ring rot was found in the field and several of the sub-lots were found positive for *Cms* infection (Table 3).

Table 3. Sensitivity of four detection methods for Clavibacter michiganensis ssp. sepedonicus among archived stem (S) and tuber (T) samples.

Sample Number	ELISA	IFAS	Cms50/72	CelA	BRR (%)
Sample 1a(S)	0/20	0/20	0/20	3/20	<1
Sample 1b(T)	0/36	0/36	0/36	6/36	<1

Summary and Conclusions:

Bacterial ring rot continues to be a sporadic disease problem in North America despite a zero tolerance regulation. Symptomless infections of *Cms* in seed lots are likely the source of ring rot for the potato industry. Reliable detection of symptomless *Cms* will aid in reducing the impact of ring rot in the future. The development of a real-time PCR format using a CelA primer has the advantage in that it only detects virulent *Cms*. Our preliminary studies indicate that the CelA primer is specific to *Cms* and that it is more sensitive in the detection of *Cms* than ELISA, IFAS or Cms50/72 PCR primers.

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BIOLOGIZED SYSTEM OF EARLY POTATO PROTECTION AGAINST COLORADO POTATO BEETLE

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The purpose of the biologized protection system is to optimize the phytosanitary state of early potato agrophytocenosis by creating conditions for proper yield and quality of potato tubers. The condition is provided by reaching the full biological potential of potato varieties through their adjustment to the environmental conditions and phytopathogen- and -pest -caused stress relief.

The pest is characterized by well developed biochemical mechanisms of insecticide detoxication which are constantly being improved during the process of its ontogenesis. Taking into account the continuing expansion of the pest's spreading area it is necessary to use chemical substances increasingly. This factor makes potato an intensively treated crop .This is why the research aimed at finding and analyzing new means and systems of potato protection proves urgent today.

The biological method of potato protection against Colorado potato beetle has become more significant than ever. It offers an advantage of maintaining ecological balance in soil and plants, non-polluting the environment with toxic substances and having neutral effect of man and animals. The biological method includes activation of local useful entomofauna of potato field, use of specialized entomophags and agents, obtained from various highly active bacteria and fungi spores, and entomophathogenic nematodes. The employment of preventive methods not connected with chemical treatment allows to store and activate natural entomophags and pathogens in Colorado potato beetle control. Also, selection of insecticides and schemes of their alternative use with minimum negative effect on useful insects and pathogens are of great significance.

Today, the most developed and effective means of biological Colorado potato beetle control are preparations obtained on the basis of entomopathogenic microorganisms. The following five preventive items are registered in Russia: Acarin (originally named agravertin), Bitoxibacillin (BTB), Colorado, Novodor and Phytoverm. These preventives are characterized by strict effect selection in comparison to chemical insecticides. As a result of the biological buffering law microorganisms do not accumulate in soil, water basins and thus do not pollute landscape. The preventive BTB employs Bacteium Bacillus thuringiensis Hl as its basis and compared to other similar preparations contains three entomocide components: spores, chrystalline endotoxin and heat-resistant exotoxin.

Table 1 gives information about the scheme according to which the field two-factor experiments were carried out. Plantings were treated at the stage of economic pest hazard – 20 larvas per shrub. 2007 saw a long and extended period of Colorado beetle pupation and larva development, so plantings were treated for the 2nd time in compliance with anti-resistant strategy of insecticide use.

According to the data presented by Andrianov A. and Andrianov D. yielding capacity of early potato in the republic of Bashkortostan (Russia) is in close and direct relation to assimilative surface of potato leaves. Tables 1 show the data of leaves surface under experiment during the period of blooming and 20 days added. The tables inform us that assimilative surface develops and functions better with the use of Bitoxibacillin and combination of Bitoxibacillin with sublethal amounts of other insecticides and application of rated amount of organic and mineral fertilizer for 30 tones of tubers per hectare.

Efficiency of agrotechnical methods used in farm crops growing is mainly indicated by the final output suitable for market. Processing of yield data has shown that the factors under analysis have significantly affected the yield of early potato in experiments. The results of the processing are given in table 2. Maximum tuber yield of early potato during the whole research period is gained in the first ten days of August under Bitoxibacillin treatment of plantings and appliance of rated amount of modern modified fertilizers for 30 tones per hectare.

As an early vegetable crop early potato has high consumer characteristics. They prove that combined use of rated portion of organic and mineral fertilizer and Bitoxibacillin (table 3) has the strongest effect on improving the quality of fresh potato tubers harvested in the early summer period.

High yield and good quality of fresh potato tubers harvested in summer are achieved thanks to anti-resistant strategy of the 4th generation pesticide use. Table 4 shows biological efficiency of insecticides tested in 2006. The data of the table state the highest biological efficiency of biologized system of early potato protection against Colorado potato beetle in the republic of Bashkortostan (Russia). The use of Bitoxibacillin and combinations of its tank mixtures with sublethal amounts of other insecticides are especially effective. Similar results were obtained in 2007. Thus, the use of Bitoxibacillin in its pure form and combinations of its tank mixtures with sublethal amounts of the 4^{th} generation other class insecticides brings about planned productivity and quality of fresh tubers harvested in summer, minimizes the resistance of Colorado potato beetle and reduces the pesticide load on agrolandscape.

Table1 Maximum leaves surface of early potato of "Nevsky" variety in relation to biologized system of protection against Colorado potato beetle, and organic and mineral fertilizing system. Experimental and training farm "Milovskoye", Federal State Educational Institution of Higher Professional Education Bashkir State Agrarian University, 2006-2007, thousand square meters per hectare

Insecticide	Fertilizer doze		
	Control	Rated portion for 20 tones per hectare	Rated portion for 30 tones per hectare
Without insecticide	19,1	26,4	28,1
Carate, 0,1l/ha	34,6	47,7	50,8
Bancol, 0,2kg/ ha	35,5	49,3	52,6
Confidor extra, 0,03 l / ha	36,4	51,8	55,3
Actara, 0,06kg/ ha	36,8	52,5	56,3
Regent, 0,02 l/ ha	35,0	48,8	52,1
Match, 0,3 l/ ha	35,9	49,0	53,7
Bitoxibacillin (BTB), 5 kg / ha	37,8	54,5	58,0
BTB, 2,0kg/ ha	36,0	50,7	53,6
BTB, 2kg/ha + Match, 0,03l/ha	36,5	50,2	55,9
BTB, 2kg + Actara, 0,006kg/ha	36,6	52,2	57,7
BTB, 2kg/ha + Bankol, 0,02kg/ha	37,7	53,0	53,2
BTB, 3kg/ha + Regent, 0,002l/ha	36,6	52,8	53,0
BTB, 2kg/ha + Confidor extra,0,003l/ha	36,4	54,1	55,2
BTB, 2kg/ha+ Carate, 0,01l/ha	34,8	48,0	54,1

Table 2 Tuber yield of early potato of "Nevsky" variety in relation to biologized system of protection against Colorado potato beetle, and organic and mineral fertilizing system. Experimental and training farm "Milovskoye", Federal State Educational Institution of Higher Professional Education Bashkir State Agrarian University, 08.08.2006-2007, tones per hectare

Insecticide	Fertilizer doze		
	control	Rated portion for 20tones per hectare	Rated portion for 30tones per hectare
Without insecticide	9,4	13,9	15,0
Carate, 0,1l/ha	21,4	30,1	32,8
Bancol, 0,2kg/ha	22,9	32,8	35,9
Confidor extra, 0,03l/ha	22,9	32,7	32,8
Actara, 0,06kg/ha	22,8	32,7	35,8
Regent, 0,02l/ha	22,9	32,7	35,8
Match, 0,3l/ha	22,7	32,6	35,6
Bitoxibacillin (BTB), 5kg/ha	23,2	33,3	36,4
BTB, 2,0kg/ha	22,8	32,7	35,9
BTB, 2kg + Match, 0,03l/ha	22,8	32,6	35,7
BTB, 2kg/ha + Actara, 0,006kg/ha	22,9	32,8	35,9
BTB, 2kg/ha + Bancol, 0,02kg/ha	22,9	32,9	36,0
BTB, 3kg/ha+ Regent, 0,002l/ha	22,7	32,6	35,6
BTB, 2kg/ha + Confidor extra,0,003l/ha	22,8	32,7	35,8
BTB, 2kg/ha + Carate, 0,01l/ha	22,9	32,9	36,0
LSD_{05}, t/ha	1,5		

Table 3 Tuber quality of early potato of "Nevsky" variety in relation with biologized system of protection against Colorado potato beetle, and organic and mineral fertilizing system. Experimental and training farm "Milovskoye", Federal State Educational Institution of Higher Professional Education Bashkir State Agrarian University, 08.08.2006

Insecticide	Dry substance content, %	Starch content, %	Vitamin C content, mg/%	Nitrate content, mg/kg	Output, %
Rated portion for 30 tones per hectare					
Without insecticide	19,5	12,5	13,6	146	58,8
Carate, 0,1l/ha	24,9	17,2	19,1	75	94,2
Bancol, 0,2kg/ha	24,9	17,2	19,1	72	94,4
Confidor extra, 0,03l/ha	25,0	17,3	19,2	75	94,4
Actara, 0,06kg/ha	25,0	17,3	19,2	73	94,4
Regent, 0,02l/ha	24,9	17,2	19,1	75	94,4
Match, 0,3l/ha	25,0	17,3	19,3	73	94,4
Bitoxibacillin (BTB), 5kg/ha	25,0	17,3	19,3	72	94,4
BTB, 2,0kg/ha	25,0	17,3	19,3	73	94,4
BTB, 2kg + Match, 0,03l/ha	25,0	17,3	19,2	73	94,4
BTB, 2kg/ha + Actara, 0,006kg/ha	25,0	17,3	19,2	72	94,4
BTB, 2kg/ha + Bancol, 0,02kg/ha	25,0	17,3	19,2	73	94,4
BTB, 3kg/ha+ Regent, 0,002l/ha	25,0	17,3	19,3	73	94,4
BTB, 2kg/ha + Confidor extra,0,003l/ha	25,0	17,3	19,3	72	94,4
BTB, 2kg/ha + Carate, 0,01l/ha	24,9	17,2	19,2	75	94,4

Table 4 Biological efficiency of biologized system of protection against Colorado potato beetle, and organic and mineral fertilizing system related to larvas. Experimental and training farm "Milovskoye", Federal State Educational Institution of Higher Professional Education Bashkir State Agrarian University, %

Insecticide	Fertilizer doze								
	Control –without fertilizers			Rated portion for 20 tones per hectare			Rated portion for 30 tones per hectare		
	Control dates			Control dates			Control dates		
	In 3 days	In 7 days	In 14 days	In 3 days	In 7 days	In 14 days	In 3 days	In 7 days	In 14 days
Without insecticide	39,2	42,9	39,1	44,6	49,0	44,6	47,5	52,2	47,6
Carate, 0,1l/ha	82,2	98,3	98,6	82,4	98,6	98,6	82,6	98,7	98,8
Bancol, 0,2kg/ha	93,2	98,2	98,6	93,4	98,6	98,8	93,5	98,8	98,8
Confidor extra, 0,03l/ha	84,7	96,4	99,4	85,1	97,7	99,6	85,3	97,5	99,7
Actara, 0,06kg/ha	92,6	100,0	96,3	93,0	100,0	96,7	93,2	100,0	97,0
Regent, 0,02l/ha	94,6	100,0	100,0	94,7	100,0	100,0	94,9	100,0	100,0
Match, 0,3l/ha	86,4	98,8	100,0	86,6	99,2	100,0	86,8	99,6	100,0
Bitoxibacillin (BTB), 5kg/ha	78,8	98,6	99,6	81,4	99,1	99,6	82,2	99,4	99,8
BTB, 2,0kg/ha	68,4	92,3	99,2	68,5	93,4	99,2	68,8	93,7	99,2
BTB, 2kg + Match, 0,03l/ha	68,6	93,6	99,6	69,1	94,3	99,8	69,2	94,6	99,8
BTB, 2kg/ha + Actara, 0,006kg/ha	73,5	96,1	98,3	74,1	96,5	98,7	74,4	96,7	98,9
BTB, 2kg/ha + Bancol, 0,02kg/ha	89,8	99,4	100,0	90,2	99,5	100,0	90,6	99,8	100,0
BTB, 3kg/ha+ Regent, 0,002l/ha	80,6	98,8	100,0	81,7	99,4	100,0	82,7	99,6	100,0
BTB, 2kg/ha + Confidor extra,0,003l/ha	69,8	93,7	100,0	70,2	94,6	100,0	70,4	95,1	100,0
BTB, 2kg/ha + Carate, 0,01l/ha	78,4	93,6	99,6	78,7	94,2	99,8	79,1	94,8	99,8

EFFECTS OF INTERACTIONS BETWEEN TWO APHID SPECIES ON BEHAVIOURAL RESPONSES AND PERFORMANCE OF THE POTATO APHID *MACROSIPHUM EUPHORBIAE*

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Induced plant response has become an increasing area of research through the two last decades. Phytophagous insects may elicit a wide range of plant defence responses following their attack which can durably modify host plant quality. Thus, insect herbivores may be affected from attacks previously done by other individuals, and indirectly compete via a shared host plant. *Myzus persicae* et *Macrosiphum euphorbiae* are important potato crop (*Solanum tuberosum*) aphid pests that may simultaneously colonize the same plant in open fields. This observation led us to examine intraguild interactions between these two phloem-sap feeders.

The systemic effects of previous infestations of *S. tuberosum* plants by *M. persicae* or *M. euphorbiae* were assessed on orientation and feeding behaviour, and performance of *M. euphorbiae*. Dual choice behavioural assays realised in a darkened area and electropenetrography (DC-EPG) experiments were performed to evaluate the effects of a previous infestation of the plant on aphid orientation and feeding behaviour, respectively. Larval mortality, pre-reproductive period and fecundity were measured every third day to assess previous infestation influence on aphid performance.

Behaviour and performance of *M. euphorbiae* were affected by conspecifics pre-infestations. When tested versus a blank (no plant odour), aphids were attracted by the odour of their host plant whatever non-infested or pre-infested by conspecifics or heterospecifics.
A non-infested plant was preferred to a plant pre-infested by conspecifics. Furthermore, electropenetrographs revealed that the total duration of phloem sap ingestion was reduced by 60 % on plants pre-infested by conspecifics. However, such a reduction in the ingestion phase did not induce any alteration of aphid development.

On the other hand, plants pre-infested by *M. persicae* were significantly preferred to both non-infested plants and plants pre-infested by conspecifics. Heterospecific pre-infestation did not modify the feeding behaviour of *M. euphorbiae*. Conversely, several parameters assessing aphid performance were improved by conspecific pre-infestation, as pre-reproductive period and intrinsic rate of natural increase.

Such modifications of host plant colonisation behaviour in *M. euphorbiae* could be due to a systemic induced response, reflecting chemical and/or physical changes in potato plants challenged by aphids. These results suggest that aphids might discriminate between plants expressing different responses according to biotic and abiotic stresses and that they adapt their colonisation behaviour. In open fields, alate aphids would migrate from a plant to another until they settle their colony on a healthy plant, leading to virus dispersion within potato fields.

EFFECTS OF MINERAL OIL ON THE POTATO APHID MACROSIPHUM EUPHORBIAE

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Aphids are major pests of potato crop, essentially because they transmit numerous viruses that can cause up to 20% yield losses. Weekly sprays of mineral oil represent the only relevant method to prevent non persistent virus transmission to potato fields. However, despite its wide use, mechanisms underlying plant protection to virus transmission by oil spray are still poorly understood.
Different hypotheses have been proposed and studied in the present work: (i) mineral oil spray acts by direct aphid intoxication, (ii) spraying potato induces plant response leading to aphid history traits alteration or (iii) plant treatment affects aphid colonization especially orientation and feeding behaviour.

To test these hypotheses, physiological and behavioural experiments were performed on the potato aphid, *Macrosiphum euphorbiae*.
Direct effects of oil treatment on aphid's physiology were assessed by measuring daily mortality and fecundity rates. Topical application and inhalation of oil volatiles at different concentrations were realised. As mineral oil has been reported to reach phloem elements crossing plant tissues, oil at different concentrations was delivered to aphids by mean of artificial diet. The aphids were confined in clip cages to assess effects of plant treatment on insects physiology. Nymphal survival, prereproductive period and fecundity rates were checked to calculate the intrinsic rate of population's natural increase. Oil sprayed plants were also used to evaluate treatment effects on aphid orientation and feeding behaviour using Y olfactometer and electrical penetration graph techniques, respectively.
Results showed that topical application of mineral oil had a lethal effect on aphids. Plant treatment by spraying mineral oil increased nymphal mortality, while fecundity of surviving insects was enhanced. Behavioural assays showed that plant treatment inhibited potato foliage attractiveness to aphids for at least during 24 hours, whereas feeding behaviour was slightly modified.

These results lead to suggest that oil treatment effectiveness in preventing PVY transmission by aphids to potato plants would rely on other mechanisms than direct effects on aphid behaviour and physiology.

Further trials will be carried out in order to assess if oil treatment could alter virus acquisition and/or transmission by aphids or induce plant defense response against virus.

Phytophthora

EVOLUTION OF THE POPULATION OF PHYTOPHTHORA INFESTANS IN FRANCE. EPIDEMIOLOGIC AND PHENOTYPIC MARKERS

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Keywords : *Phytophthora infestans*, late blight, mating type of strain, fungicide resistance, epidemic, aggressiveness of the pathogen.

Since 1993 the Plant Protection Service carries out a survey in France on the strains (isolates) of Phytophthora infestans. The mating type and fungicide resistance (metalaxyl) are determined.
Before 2003, there was no A2 strain, metalaxyl resistance was present with some variation but very often below than 40 % of the samples.
Since 2003 in North of France, there is a real evolution, with detection of A2 strains and a regular increase until a high level in 2007 (80%).

The metalaxyl resistance seemed to increase since 2003 to 2006, but it is stabilized in 2007.

The results in others regions of France are different.

The tests applied on others fungicides show no resistance phenomena.

On an other part, the Plant Protection Service advises the growers by two tools : warning system and a DSS. The systems use epidemiological models but also the scouting in fields and around. The observed epidemics seem to be more previous and more severe but the most important cause remains the climatic conditions, even if a new aggressiveness cannot be completely discarded. At this moment we have a lot of questions:

Origin of the phenomena : seeds ? climate ? aggressiveness of strains ... ?

Consequences : oospores, earlier outbreaks ?

Even if we have no answer, we modify our control strategy.

INTRODUCTION

The most important assignment of the Plant Protection Service is to take on the sanitary supervision of the territory: quarantine organisms but also others enemies of the crops.

There are several targets :
- to find very early emergent phenomens : new pathogens, evolution of the pathogens (new strains, fungicide resistance)., so a lot of sample are analysed in the laboratory.
-to take very early adapted control methods
-to use the observations to complete the evaluation of risks by epidemiological models in order to set up advises to the growers (warning system)

MATERIALS AND METHODS

"Bio vigilance" survey is programmed with protocols for sampling and analyses .
For the samples , the technican takes a lot of necroses in the fields or dumps (more than 15 necroses).
The resistance test to mefenoxam follow the method described by the FRAC (Fungicide Resistance Action Comitee) using the floating leaf disc method : there are three doses of fungicide : 0,1-10 et 100 ppm, with 2 replications for each concentration, each disc is inoculated with a droplet of the tested isolate inoculum. The condition of test is an incubation at 16°C with photoperiod 16h/8h during 7 days. The Ec_{50} (concentration which inhibits 50 % of the sporulation) is determined graphically. The resistance level is determined with the scale :
$Ec_{50} < 0.1$ ppm : sensitive isolate, $0.1 < Ec_{50} < 10$ ppm : intermediate isolate,
$Ec_{50} > 10$ ppm : resistant isolate

The determination of mating type is tested by a confrontation of mycelium plugs from the isolate with reference strains A1 and A2 on agar medium. The incubation is realised at 16°C during 10 days in darkness The observations are made under microscope at the border of the confrontation line to detect the possible presence of oospores.
The oospores formation requires the two opposite mating types.

RESULTS AND DISCUSSION

Mating type

Since 1993, more than 2 000 samples have been analysed to determine the mating type and fungicide resistance, particularly mefenoxam (metalaxyl).
A2 strains were not detected except few cases(3) in fields in 1997(year with severe epidemic) and in gardens on potatoes and tomatoes.

In 2003, we have detected A2 in 6 % of the samples, the phenomen has increased very quickly in North of France in 2004, 2005,2006 and 2007 : A2 strains mean :
30 % of samples in 20 % of fields in 2004, 41 % of samples in 39 % of fields in 2005, 74 % of samples in 79 % of fields in 2006, 76 % of samples in 73 % fields in 2007 (Fig.1). The A2 strains are now detected on different types of plants : dumps, volunteers, gardens .
(cf example : Tables 1 an 2).

In the others regions A2 strains are also detected but at different levels (fig2), and in each region, the A2 strains increase :

Brittany : before 2005 only few A1 strains (INRA information), 2005 : 4 % of the sample, 9% in 2006 and 42% in 2007.

In Alsace, there is 68% A2 in 2005, 58% in 2006 and 79% in 2007.
In Center of France, the mating type is exclusively A2. In the region of South West, 25% of strains are A2 in 2006 and 70% are A2 in 2007.

Mefenoxam (metalaxyl) resistance

The resistance to metalaxyl is detected since 1981, the second year of use of this compound in France.

After, 1981, a catastrophic year with lot of symptoms in fields due to resistance (initial inefficacy on some strains), the strategy of mixture in commercial products and strategy of sprays (preventive sprays, short interval between two sprays, limitation of number of treatments) the fungicide with metalaxyl or mefenoxam have been use with success.

During these years, metalaxyl resistance have been detected at different levels, very often below than 50 %.

Since 2004, the resistance seems to increase in North of France in fields (until 70 % of the samples) and on dumps (until 45 % of the samples).

This last aspect is very important and perhaps dangerous, because it is the state of the primary inoculum (fig.3, fig.4)

In the others regions, metalaxyl resistance is also detected at different levels, with a relative stability in Brittany (80 % - 60 %), a quick increase in South West and Alsace (15 % in 2005 to 80 % in 2007).

Epidemic evolution

Since 2004 the growers have met season with high difficulties to control the disease particularly in 2005 (North of France) and 2007, also in 2006 but with not so many problems.
In 2005 and 2007, the start of the epidemic was very early (5-10 of May) and there was a high increase of the disease during July 2005 with many symptoms in the fields (Table 3).

In the first part of July several cultivars lost the late blight resistance

In 2006, the emergence of the crops was delayed until the end of May and immediately some atypical symptoms appeared on isolated plants.

But the most important information is that the forecasted risks given by the models (with “old biological” data : maximun of risks of contamination according to Guntz Divoux abacus : 19 hours of 90 % of hygrometry at 12°C, 13 hours of hygrometry at 20°C, shortest latence period of Guntz Divoux Concé between 16,6°C Tm to 20°C Tm (Tm : average day temperature), maximun of sporulation between 18°C to 22°C) were good except in some cases of very early risks in 2007.

So, there are many questions about the cases. New strains more aggressive ? milder climate ?

CONCLUSION AND QUESTIONS

Evolution on the strains

Since 2003, the population of *Phytophthora infestans* have changed with new strain, it is confirmed by genotypic studies (Didier Andrivon, INRA.; U. Gisi, Syngenta; David Cooke, SCRI)

The first researches of Didier Andrivon show that, on samples from North of France in 2004 and 2005, the A1 strains are a little more aggressive than A2 strains. Doctor U. Gisi (Syngenta) has compared isolates from North of France in 1997, 2006 and 2007. The results are : no A2 strain in 1997, but, 80 % and 76 % of A2 strains in 2006 and 2007. He finds no difference of aggressiveness between A1 and A2 strains on 2006 samples. He finds that the 1997 isolates are a little more aggressive than the isolates of 2006.

So, why this evolution of *Phytophthora infestans* in France since 2003 if there is no difference of aggressiveness between A1 and A2 strains ?

Is important the origin of the importation of seeds ? The North of France have imported more seeds from The Netherlands than Brittany for example. Does the proximity of regions take a role : North near The Netherlands, Alsace near Germany ?

Does the evolution of the climate conditions with softer temperature, influence this evolution?

Does the agronomic practices (choice of cultivars, earlier plantation) take some importance ?

Why the disease seems to be more difficult to control and the epidemic more severe ?

Evolution of the epidemic

The new strains A2 don't seem to be more aggressive.

The models (with "old" data of biology) are always correct except in some cases of early risks.

The most important cause seems to be a softer climate with consequence particularly in :

Winter : if there is no frost, the initial inoculum is more important

April : there are earlier plantations, and faster growth

May: the production of spores on dumps is probably higher

June and July : the epidemic is more rapid because there are more numerous periods with risks (T° 12 °C to 20°C)

Evolution of our advices to the growers

For the tools, we don't change the data of our epidemiological model and DSS but we decide to start the use from January (until now it was the first of April because there was some frost until this date, it was not the case in 2007), so we will measure the global epidemiological risks including dumps.

We will also have a more intensive survey of the durability of cultivars resistance.

According to the advices, we will change the fungicides strategy except for the products with mefenoxam.

The mefenoxam strategy in 2008 will be : only preventive sprays at the beginning of the season, never after the first of July, with only 7 days between two sprays, preferable 1 phenylamid per year. We will advise to stop the sprays with phenylamids, if symptoms of late blight are detected around the fields and if the metalaxyl resistance is detected on many samples on dumps. We will advise the growers to be very careful early in the season, because the crops will come back in North of France in fields with A2 strains in 2003, 2004, (2005) so with potential risks of oospores (but not demonstrated). We will insist on early sprays with adapted fungicide if the models and Decision Support System indicate some risks.

ACKNOWLEDGEMENTS

Thakks to Docteur Didier Andrivon (INRA Rennes) France, David Coole (SCRI Scotland), Docteur U.Gisi(Syngenta) for oral informations and scientifics exchanges .

Fig. 1. : Mating type 2003-2007 (Fields : crops and volunteers, dumps, gardens) North Pas de Calais, Picardy, Champagne Ardenne, Normandy

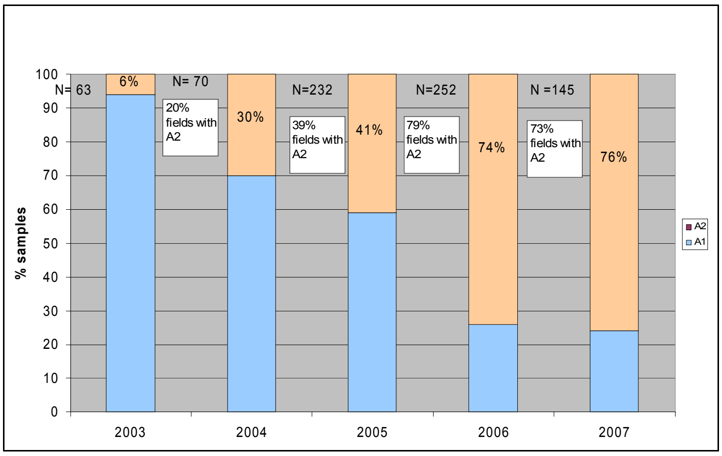


Fig. 2. : % A2 by region in 2006 ( ), 2007 __

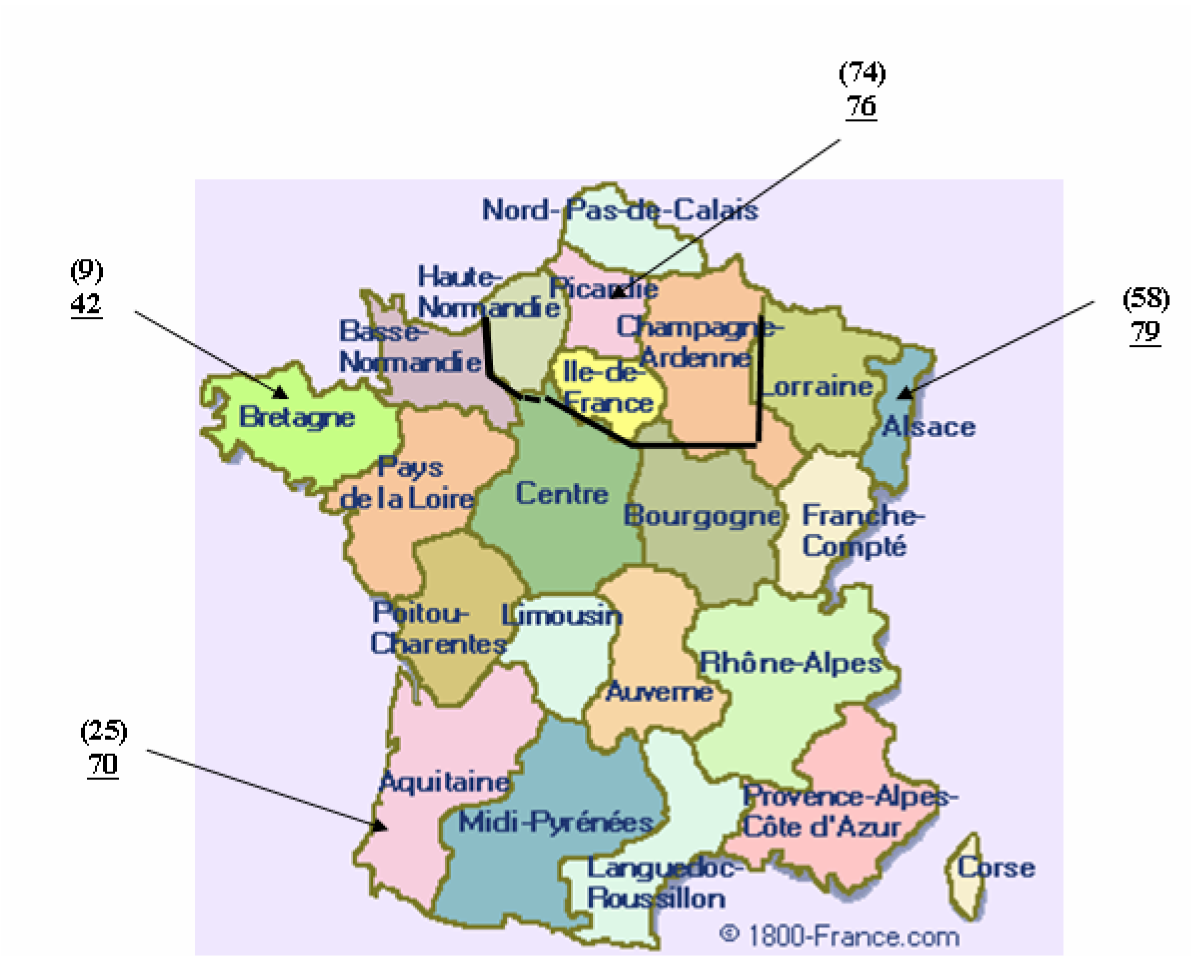


Fig. 3. : Mefenoxam resistance fields : crops and volunteers, dumps, (gardens) (north Pas-de-Calais, Picardy, Champagne Ardenne, Normandy) 2004 to 2007

N = number of samples

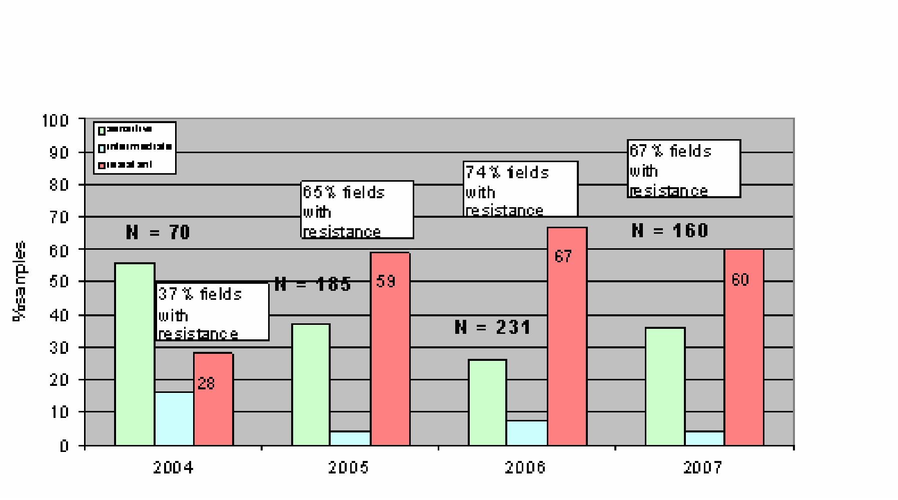


Fig.4. : Mefenoxam resistance on dumps (North Pas-de-Calais, Picardy, Champagne Ardenne, Normandy) 2004 and 2007

N = number of samples

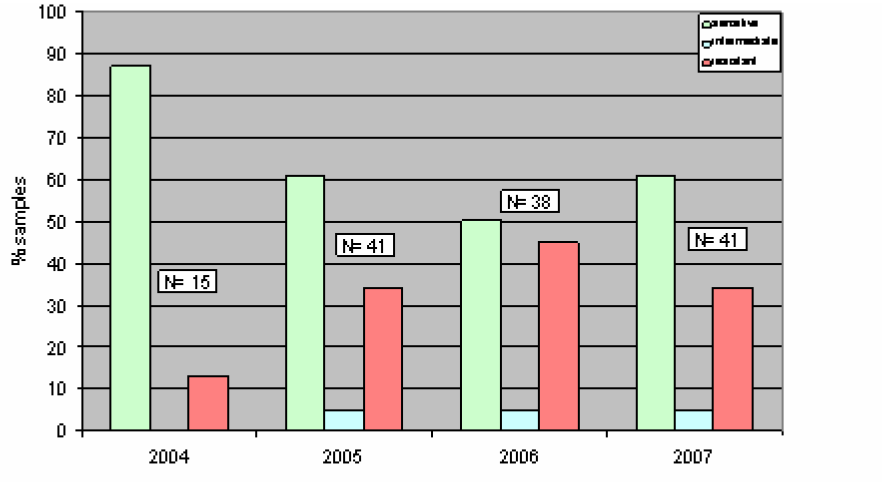
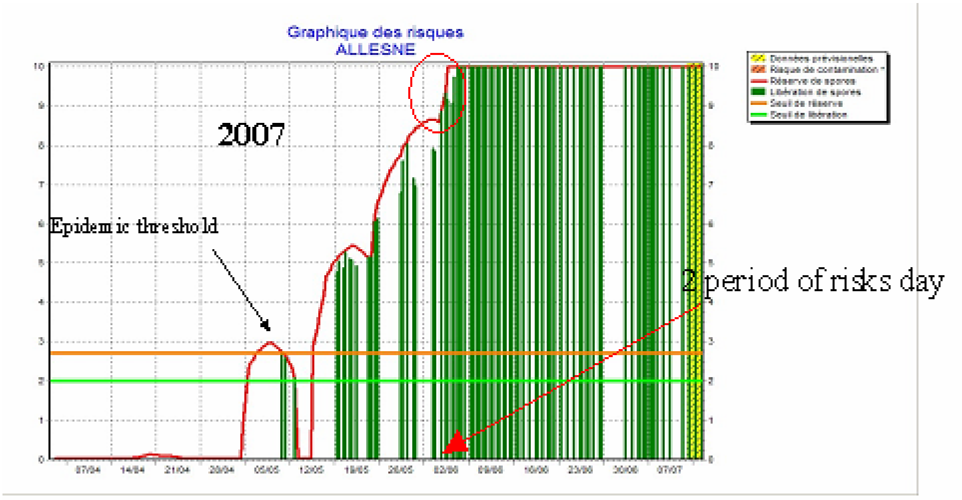


Fig. 5. Exemple of risk give for the DSS MILPV in 2007



The maximum risk is reached of June and it is maintained during June

Table 1 : Mating type in North of France 2006

	A1	A2	A1/A2
Field	48 (32 fields, 5 with A1 and A2)	151 (109 fields, 5 with A1 and A2)	1 (1 field)
Garden tomato	1	5 (1 garden)	
Garden potatoes		6 (2 gardens)	
Dumps	14 (12 dumps, 6 with A1 and A2)	24 (12 dumps, 6 with A1 and A2)	2
TOTAL	*63*	*186*	*3*

252 samples, analysed for the part « North » of France
74 % strains are A2 (79% fields with A2)

Table 2: Mating type in North of France 2007

	A1	A2	A1/A2
Field	30 (17 %)	81 (73%)	0
Garden		6	1
Dumps	5 (18 %)	23 (82 %)	
TOTAL	*35 (24 %)*	*110 (76 %)*	*1*

Table 3 : The epidemics in 2004, 2005, 2006, 2007 in North of France

Years	Severety	First symptoms	Epidemic Threshold on susceptible cultivars	Periods risks	Control	Model and DSS forecast	Questions
2004	Moderate	End of May on dumps and volunteers	(May 20) June 17	some risks mid of July	easy and good success	Correct	
2005	"usual" in France very severe in North of France	May on dumps	early 8 of May	5 of July -->July	Very difficult in July high risks difficulties of sprays rainfastness growth of crop cultivar resistance "broken" many symptoms in fields	Correct	Climatics conditions aggressivess ? metalaxyl resistance not proved
2006	Relatively severe	Beginning of May on dumps	25 of May	End of May August	Some symtomps on isolate plant during difficult emergence en of May August : difficult (rain) some symptoms on tubers	Correct	Contamination in may → seed origine ? (oospore ? Probably not
2007	The most severe since 10 years in all regions of France	25 May on dumps	10 of May	Since June very severe in July	Very difficult in July with lot of symptoms mong sprays risks every days and rain every days	Correct Excepted some case of early risk	Many sources of contamination climat : no cold in winter, April very soft aggressiveness ?

RECENT GENETIC CHANGES IN THE LATE BLIGHT POPULATIONS IN USA

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INTRODUCTION

Late blight, caused by the oomycete pathogen *Phytophthora infestans* (Mont.) de Bary, has become an increasingly important problem to agriculture in the United States and many other countries in the past decade. More aggressive, fungicide-resistant and host-specialized isolates have appeared attacking potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* Mill.) crops. In the United States, the pathogen has been reported as being more genetically diverse on tomato than on potato (Wangsomboondee *et al*., 2000). Since the migrations of *P. infestans* from its presumed center of origin, Mexico, from the late 1970s on, major population displacements and genetic changes in populations outside Mexico have occurred (Goodwin, 1993). The presence of these new genotypes resulted in renewed research on the pathogen since it might now reproduce sexually due to the introduction of the A2 mating type, because new populations demonstrated increased aggressiveness (Fry, 1997). The occurrence of phenylamide (metalaxyl) resistant isolates further added to the devastation and control problems associated with the new populations (Goodwin, 1994). Therefore, it is important to monitor changes in *P. infestans* populations continuously and to develop integrated management strategies for late blight control.

MATERIALS AND METHODS

Collection and culture of isolates. Blighted material was collected in the major production areas in the US by cooperators during crop inspections of naturally-infected fields (Table 1). Each sample consisted of infected leaves and stems from a single crop. Information on site, fungicide usage, and potato or tomato cultivar and blight incidence was collected for each sample. Samples of single lesion isolates were incubated under high humidity for 24-48 h to encourage sporulation then isolates were obtained by collecting sporangia from infected foliage and initially maintained on detached glasshouse-grown potato leaflets or tuber slices of susceptible cultivars free from R-genes. Tissue pieces 3-4 mm^2 were cut from the margins of lesions, surface sterilized and transferred to Petri plates containing rye A amended with antibiotics.

Mating type determination. Unamended rye agar plates were inoculated with a mycelial plug of the test isolate, and a plug of a reference *P. infestans* isolate of either the A1 or the A2 mating type placed 20-30 mm away (four plates for each test isolate, two with different A1 reference isolates and two with different A2 reference isolates). The dual cultures were incubated at 15°C in darkness for 7-14 days, and then examined microscopically for the presence of oospores where the two colonies interacted.

Metalaxyl sensitivity *in vitro*. *In vitro* sensitivity to metalaxyl (as 'Ridomil 2E', Syngenta, 25.1% w/w metalaxyl) was assessed by comparing radial mycelial growth on rye agar amended with 10 mg metalaxyl l^{-1} to growth on metalaxyl-free controls (mean of three replicates). Isolates were designated as metalaxyl-resistant if growth was >60% of the control, -intermediate if growth was 10-60% of the control or –sensitive if growth was <10% of the control, using the criteria of Shattock (1988).

Allozyme assays. Genotypes at two polymorphic allozyme loci, *Gpi-1* (glucose-6-phosphate isomerase, GPI, E.C. 5.3.1.9.) and *Pep-1* (peptidase, PEP, E.C. 3.4.3.1.), were determined using the protocols of Goodwin *et al.* (1995). The genotypes of unknown isolates were determined by comparing their banding patterns with those of reference isolates kindly provided by cooperators.

Identification of mitochondrial DNA haplotypes. Mitochondrial DNA (mtDNA) haplotypes of isolates were determined by PCR-RFLP using a modification of the method of Griffith and Shaw (1998). Two-week old mycelium, grown in pea broth supplemented with 2 g $litre^{-1}$ calcium carbonate and 0.05 g $litre^{-1}$ β-sitosterol, was lyophilized following the methods of Goodwin *et. al*. (1995) DNA extraction was performed using the Qiagen DNeasy Plant Mini Kit. DNA was amplified using two pairs of oligonucleotide primers F2/R2 and F4/R4 synthesized by GibcoBRL®Life Technologies (Gaithersburg, MD), according to the sequences given by Griffith and Shaw (1998).

RG57 Fingerprinting. DNA fingerprinting using the moderately repetitive probe RG57 was carried out as described by Goodwin *et al.* (1992) except the RG57 insert was PCR amplified with the oligonucleotide primers M13 (forward and reverse).

RESULTS AND DICUSSION

Single isolates of *P. infestans* were recovered from symptomatic potato and tomato foliage in the 1995, 1998 and 2005 outbreaks in Alaska (Table 1). The 1998 and 2005 outbreaks were similar because both proved to be caused by the relatively aggressive US-11 allozyme genotype (Table 2) and had significant economic impact for commercial potato growers. All of the isolates from potato and tomato in 2005 displayed the allozyme pattern associated with the US-11 genotype, possessed the IIb mitochondrial haplotype, and were mating type A1. The 1995 outbreak was caused by the relatively rare US-7 genotype and started so late in the season that economic impact was minimal. Because late blight occurs so sporadically in Alaska, fungicides are not routinely used, but it is unlikely that the pathogen persisted locally between outbreaks.

P. infestans isolates obtained from late blighted tomato fields in New Jersey in 2003 were all A2 mating type, metalaxyl-resistant and mtDNA haplotype Ia. However, these isolates were homozygous at the loci coding for both glucose-6-phosphate isomerase and peptidase, having *Gpi 122/122, Pep 100/100*. RG57 analysis showed that all the tomato isolates from New Jersey had a unique and previously unreported fingerprint (Table 2). Although severe late blight symptoms were present in nearby potato crops, the isolates were typical of the US-8 genotype (Table 2). The *P. infestans* genotypes isolated from adjacent tomato and potato crops in Florida and North Carolina were quite distinct and the presence of additional RG57 bands in the fingerprints of isolates from tomato, compared with the US-8 strains from potato, suggests that the tomato strains were not derived from the potato ones.

Single-lesion isolates from late blighted tomatoes fields in Pennsylvania were all A2 mating type, metalaxyl-sensitive, mtDNA haplotype Ia and were *Gpi 100/122, Pep 100/100*, characteristics atypical of isolates of *P. infestans* from potato in the same area (Table 2), which were all of the US-8 genotype. The tomato isolates had the allozyme banding pattern and mating type associated with the US-14 genotype (Goodwin *et al.*, 1998). As with the late blighted tomatoes in New Jersey, RG57 analysis (Table 2) showed that the tomato isolates had a unique fingerprint (different from that found in the New Jersey potato isolates) which does not appear to have been reported previously.

The collation of such molecular marker data with information on mating type (thus likely sexual reproduction) and fungicide resistance is a fundamental aim of the project. This will allow us to determine the mechanisms and tempo of genetic change within populations. This consideration of the impact of new lineages on the epidemiology of late blight in the United States indicates that there is now greater diversity within the tomato population of *P. infestans* than prior to the 1990s. The new lineages differ from old lineage(s) in some but not all epidemiologically important aspects. The occurrence of several novel lineages implies that there will be some diversity in the epidemiology of the disease. The implications to pathologists are that the differences need to be identified, quantified and incorporated into disease management recommendations.

Table 1. Host origin, location, and collection date of isolates of Phytophthora infestans evaluated in this study

Hosts	Location	Years when sampled	Number of isolates
Tomato	Alaska	1995, 1998, 2005	23
Potato[1]			6
Tomato	Florida	2004, 2006	8
Potato[1]			18
Tomato	North Carolina	2006	11
Potato[1]			19
Tomato	New Jersey	2003	3
Potato[1]			11
Tomato	Pennsylvania	2004	5
Potato[1]			19

[1]Late blighted potato hosts that were located in bordering, neighboring or close-by plantings

Table 2. Mating type, metalaxyl resistance, allozyme genotype, and RG57 fingerprint of isolates of Phytophthora infestans collected from potato and tomato hosts in five USA locations

Host	*Gpi* genotype[a]	Metalaxyl sensitivity[b]	Mating type	US genotype[c]	RG57 fingerprint
Tomato (Al-05)	*100/100/111*	R	A1	US-11	1,5,9,10,13,14,16,20,21,24,25
Potato[d]	*100/100/111*	R	A1	US-11	1,5,9,10,13,14,16,20,21,24,25
Tomato (Fl-06)	*100/122*	R	A2	n/d	1,5,10,13,14,18,20,21,24,25
Potato[d]	*100/111/122*	R	A2	US-8	1,5,10,13,14,15,20,21,23,24,25
Tomato (NC-06)	*100/100*	R	A2	n/d	1,3,5,7,10,13,14,16,18,20,21,24,25
Potato[d]	*100/111/122*	I/R	A2	US-8	1,5,10,13,14,15,20,21,23,24,25
Tomato (NJ-03)	*122/122*	R	A2	n/d	1,3,5,7,10,13,14,18,20,21,24,25
Potato[d]	*100/111/122*	R	A2	US-8	1,5,10,13,14,16,20,21,23,24,25
Tomato (PA-04)	*100/122*	S	A2	n/d	1,5,13,14,18,20,21,24,25
Potato[d]	*100/111/122*	R	A2	US-8	1,5,10,14,16,20,21,23,24,25

[a]all isolates had a *Pep* allozyme genotype *100/100*
[b]Sensitivity to metalaxyl of isolates of *P. infestans*: R = Resistant; S = Sensitive; I = Intermediate (as defined by Shattock, 1988)
[c]n/d = not determined, does not conform to any published US genotype
[d]Late blighted potato hosts that were located in bordering, neighboring or close-by plantings

PHENOTYPIC TRAITS OF *PHYTOPHTHORA INFESTANS* POPULATIONS IN FINLAND AND NORTH WESTERN RUSSIA

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Introduction

Potato late blight caused by the oomycete *Phytophthora infestans* is a severe disease on potato. Under favourable conditions for the disease it can kill unprotected potato haulm in a couple of weeks. In addition rain can spread sporangia formed in potato leaves to tubers and cause tuber blight (Hannukkala *et al*. 2007). The main initial sources of late blight inoculum are considered to be infected tubers which survive to the next season in cull piles, storage or soil (Zwankhuizen *et al*., 1998). A new primary source of inoculum appeared in Europe when the old clonal lineage of *P. infestans* was replaced by a new more diverse population during the 1980s (Goodwin, 1997). The new population possesses both mating types and is able to reproduce sexually in potato in the Nordic countries (Brurberg *et al*., 1999, Hermansen *et al*., 2000, Lehtinen *et al*. 2007a). Sexual reproduction results in oospores, the resting bodies of *P. infestans,* which can overwinter in soil (Andersson *et al*., 1998, Lehtinen and Hannukkala, 2004).

The changes in phenotypic and genetic properties in *P. infestans* populations have been extensively studied in Central and Western Europe (Knapova and Gisi 2002, Day *et al*. 2004, Cooke *et al*. 2006, Sliwka et *al*. 2006), and in the Nordic countries (Brurberg *et al*., 1999, Hermansen *et al*., 2000, Lehtinen *et al*. 2007a, Lehtinen *et a.* 2007b). Population studies have been carried out at Moscow region and Far East of Russia (Elansky *et al*. 2001) but no published data is available about blight populations in North Western parts of Russia. The blight populations in Kola and Karelia are of special interest for Finland because these regions are relatively close to the eastern border of Finland and late blight has only very recently started to cause severe epidemics at the Northern regions close to the Polar Circle (Hannukkala *et al*. 2007). The aim of this study was to collect *P. infestans* isolates from Kola, Karelia and Finland and compare the phenotypic traits of these populations and their impact on blight epidemiology and disease management practises.

Material and methods

P. infestans isolates for this study were collected in 2006 and 2007 from Murmansk region in Kola, Petrozavodsk region in Karelia, Central and Northern regions of Finland. Totally 134 isolates from Russia and 236 from Finland were obtained. The Russian isolates were sampled within few days' periods in 2006 and 2007, transported across the border to Finland and mailed to MTT, Agrifood Research Finland to be tested and maintained. The Finnish isolates were collected and mailed to MTT individually during August in 2006 and 2007. The mating type of the isolates was determined on agar, response to fungicides metalaxyl-M and propamocarb-hydrochloride, and pathotype on floating leaf disks as described by Lehtinen et *al.* (2007 b). Most of the isolates were transferred into liquid nitrogen for further studies.

Results and discussion

Mating types

Both mating types A1 and A2 were present at close to 1:1 proportions in both countries in both years. The equal ratio of mating types has been typical for blight populations in the Nordic countries in 2000s (Lehtinen *et al.* 2007a and 2007b), while elsewhere mating type A2 has been less frequent or very rare (Elansky *et al*. 2001, Knapova and Gisi 2002, Day *et al*. 2004, Cooke *et al*. 2006). It is obvious that genetic diversity generated by sexual reproduction and risk of oospore derived late blight attacks early in the season must be taken into account in Kola and Karelia in blight management practises, as elsewhere in Northern Europe (Andersson *et al*., 1998, Lehtinen and Hannukkala, 2004).

Response of *P. infestans* isolates to systemic fungicides

In 2006 metalaxyl resistant isolates were not detected and percentage of intermediately resistant isolates varied between 10-20 % in Finland and Russia respectively. In 2007 metalaxyl resistant isolates appeared into the population. Their proportion in Russia was 35 % and in Finland 20 %. During early 1990s in Finland when blight control was highly dependent on metalaxyl products up to 90 % of the *P. infestans* isolates were resistant to metalaxyl (Hermansen et. *al.* 2000). When antiresistance actions during the second half of 1990s were widely adapted in blight control and novel modes of actions of fungicides were introduced, the proportion of resistant isolates in Finland decreased from 40 to 20 % from 1997 to 2000 respectively (Lehtinen et *al.* 2007b). In the beginning of the 2000s the application of metalaxyl products was almost fully abandoned and in the survey in 2003 only 2 % of Finnish *P. infestans* isolates were resistant to metalaxyl (Lehtinen et. al. 2007a). The increase of metalaxyl resistant isolates in Finland in 2007 might be due to increased knock-out use of metalaxyl fungicides in recent years. The appearance of metalaxyl resistance in the Russian blight population is difficult to explain, because metalaxyl has not been used for blight control in fields where samples were collected.

There were no isolates resistant to propamocarb-hydrochloride in Finland or Russia. The proportion on isolates tolerating low concentrations of the fungicide was approximately the same in both countries. In 2007 the proportion of fully sensitive isolates was clearly decreased in both countries in comparison to 2006. The proportion of isolates tolerating low dosages of propamocarb was at the same range as previous studies in the Nordic countries, where no failures in blight control by propamocarb products have been reported (Lehtinen et. *al*. 2007a and 2007b).

Pathotypes determined by R-gene resistance

Pathotypes in both Finnish and Russian populations could be clearly classified to those overcoming almost always 4 to 6 genes of resistances R1,3,4,7,10,11 and to those possessing the additional ability to break down one or more of the resistance genes R2,5,6,8,9. Very complex races were predominant in the Finnish and Russian populations, while in Russia isolates with less than 6 virulence factors were more common than in Finland. Pathotypes with less than 6 virulence factors have been extremely rare in the Nordic countries (Lehtinen et *al*. 2007 a) while in Russia 20 % of isolates possessed less than 6 virulences.

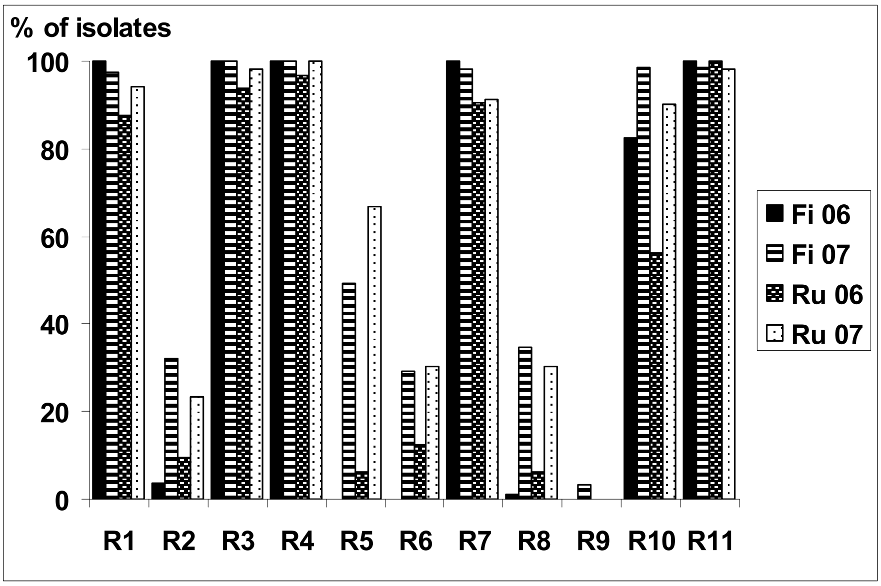

Figure 1. The prevalence of virulence factors overcoming R-genes R1 to 11 in the Finnish and Russian potato late blight populations in 2006 and 2007. (Fi= Finland, Ru=Russia, 06= year 2006, 07= year 2007).

The populations in both Finland and Russia were changed considerably more divergent in pathotypes from 2006 to 2007. This is also true for Finnish population in comparison to earlier surveys. In the average Finnish isolates contained 5.9 virulence factors in 2006 as was the case in 1997-2000 and 2003 (Lehtinen et. al. 2007 a and 2007 b) and 7.4 virulence factors in 2007 The Russian isolates possessed 5.6 virulence genes in 2006 and 7.2 in 2007. In 2006 only four different pathotypes were found in Finnish blight population while 10 different pathotypes were present in the Russian one. The population structure in 2007 was totally different from 2006. There were 27 different pathotypes in Finnish and 32 in Russian blight population.

The most common pathotype in both years and countries contained virulence factors 1,3,4,7,10,11. The same pathotype has been dominating in the Nordic in countries in 1997-2007 and 2003 (Lehtinen et. al 2007 a and 2007 b). The second common pathotype (1,3,4,7,11) in 2006 had been replaced by more complex pathotype (1,3,4,5,7,10,11) in 2007. In 2007 one Finnish isolate contained all eleven virulence genes. Two percentage of isolates in 2007 in both countries contained all virulence genes except virulence 9. The proportion of isolates containing rare virulence genes 2,5,6 and 8 was considerably increased from 2006 to 2007 in both countries (Figure 1). Virulence gene 9 was detected for the first time in the Nordic countries (Lehtinen et *al.* 2007 a) while it was not yet found in Russia.

Conclusions

The Finnish and Russian potato late blight populations in 2006 and 2007 were very similar in phenotypic traits and very similar to those in the Nordic countries in 2003. Nordic regions up to the Polar Circle and beyond have sexually reproducing populations, which means increasing genetic diversity in population and increasing risk of oospore derived late blight attacks very early in the season. High incidence of very complex pathotypes may result in a very rapid breakdown of leaf blight resistance among current potato cultivars, which is very crucial in the Russian regions, where chemical blight control is not a common blight management practice. Indications of increased metalaxyl resistance must be taken into account in future choice of fungicides. It is important to be aware of changes in population properties when planning future actions in potato late blight management in both countries.

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ANALYSIS OF GENETIC VARIABILITY BY MOLECULAR MARKERS IN TEN ACCESSIONS OF *PHYTOPHTORA INFESTANS* FROM ROMANIA

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Summary. Ten accessions of *Phytophtora infestans* were isolated, from different county of Rumania, on rye medium. For analysis of genetic variability of *Phytophtora infestans* there were used several molecular types of markers: RAPD, ISSR, PCR and SSR at the level of genomic DNA as well as PCR marker at the level of mitochondrial DNA. Following the cloning and the sequencing of the monomorphic bands from two accessions specific primers for *Phytophtora infestans* were synthesized, succeeding the conversion of RAPD markers in more specific SCAR markers. The *Phytophtora infestans* accessions were, also, characterized from the point of view of mating type and metalaxyl susceptibility.

Key words: *Phytophthora infestans,* molecular markers, mating type, metalaxyl susceptibilities, potato late blight.

Introduction.

Revealing of the genetic variability of local populations of *Pytophthora infestans* is a crucial step for an efficient potato late blight control. At the moment is difficult to perform an accurate identification of intraspecific variation of *Pytophthora infestans* by morphological feature. RAPD molecular markers could be beneficial for revealing genetic variation of this species but, in spite of its simplicity, the method has several limits. The repeatability of this technique could be a problem. Contamination with DNA from other fungi or insects, originated in the used tissue for DNA isolation, could generate a false molecular polymorphism. Also the number of amplification products obtained by RAPD being rather small, the chance of identification of genetic differences among different accessions is quite reduced and it is necessary to use many primers. On account of these limits we followed two main objectives: increasing of molecular polymorphism in order to better characterize different Romanian *Phytophthora infestans* accessions and conversion of RAPD markers into SCAR markers in order to increase the efficiency of identification of *Pytophthora infestans* in the infected material.

Material and method

For analysis of the genetic variability there were isolated, on selective rye medium with antibiotic, ten accessions of *Phytophtora infestans* from different counties of Romania, (table 1). After DNA isolation, using Roger and Benedich, (1994) protocol, there were used, for molecular analysis, several types of molecular marker: PCR, SSR RAPD and ISSR, looking for primary molecular polymorphism or, after digestion with restriction enzymes, for secondary molecular polymorphism (CAPS marker). Relying on RAPD molecular polymorphism, genetic distances and phenetic relationship among accessions were established. We tried to increase the RAPD molecular polymorphism by digestion of amplification products with restriction enzymes (Hae III, Rsa I, Not I, Hind III, Hinf 1). Establishing of mating type was done by hybridisation on rye medium of our accessions with the received A1 and A2 mating type from Wageningen University, Laboratory of Phytopatology. Metalaxyl susceptibility was determinate by classical methods (Shattock,1988).

Table 1. Different accessions of potato late blight from Romania

Symbol of accessions	Accession derived from potato variety	Romanian location of accessions
77	Kondor	Criseni ,Salaj county
79	Kondor	Cociu, Bistrita-Nasaud county
83	Sante	Hida, Salaj county
88	Desiree	Satu Lung, Cluj county
89	Laura	Buciumi, Maramures county
68	Princesse	Braniste, Dambovita county
72	Sante	Letca, Salaj county
73	Sante	Odorhei, Salaj county
91	Desieree	Satu Lung, Cluj county
98	Red Sec	Dorohoi, Botosani county

Results

PCR specific primers, like ipiB-1F/ipiB-1R, recommended by Perez et al.,(2001) ; ITS-3/ITS-4; ITS-3/PISP, primers based on sequence analysis of internal transcribe spacer region of ribosomal DNA (Tooley et al.,1997; Lee,2001), gave no any polymorphism among the ten accessions, with or without digestion of amplification products by restriction enzymes.

Microsatellite markers, recommended by Lees at al., (2006), also gave no polymorphism among the ten accessions.

The amplification products obtained from different accessions by RAPD show a significant polymorphism in relation with the used primer. From the thirteen decamer primers used, two gave the best results (OPC-9 and OPC-20), the polymorphism obtained in some accessions being obvious and stable.

Nevertheless the heterogeneity revealed by dendrogram was quite reduced four accessions being monomorphic (83,68, 72 and 73 accessions).

By digestion of amplified RAPD products with the five restriction enzymes the heterogeneity increased significantly.

The ISSR markers gave similarly result as RAPD markers, the amplification product being many more.

Analysis of secondary molecular polymorphism at the level of amplified mitochondrial DNA with H4 pair of primers, digested by EcoRI restriction enzyme (CAPS markers), revealed a genetic differentiation among Rumanian *Phytophthora infestans* accessions (figure 1). On this basis there were established that the ten *Phytophthora infestans* accessions belong to two haplotypes, one accession belongs to Ia haplotyp and the other nine belong to IIa haplotype (Griffith and Shaw, 1998; Botez et al.,2007)). There is one opinion which consider that mating type is under the control of mitochondrial genes (Huan and Hsiung, 2005).

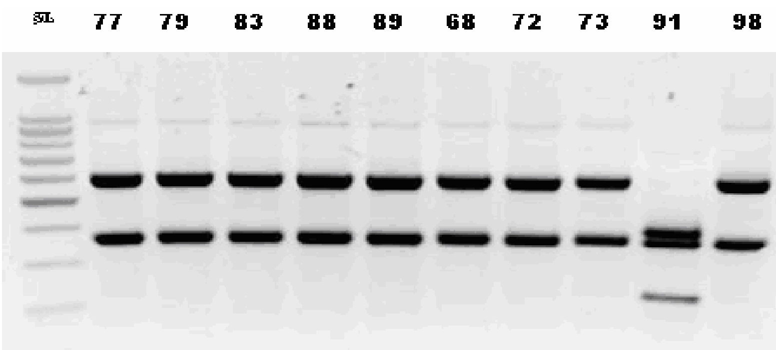

Figure 1.Restriction fragments from digestion of H4 amplification products with EcoR I restriction enzyme, in ten Phytophthora infestans accessions. SL.- Smart ladder.

Following the cloning and the sequencing of some monomorphic RAPD bands from two accessions (77 and 79 accessions), there were synthesized specific primers for *Phytophtora infestans*, succeeding the conversion of RAPD markers in more specific SCAR markers (figure 2).

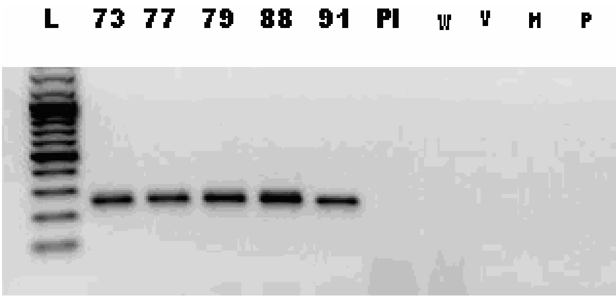

Figure 2. Products of amplifications obtained with specific primers, derived from a monomorphic cloned band of 79 accession, in five Phytophthora infestans accessions and in plasmidial (Pl), wheat (W), vine (V), maize (M) and potato (P) DNA

Now we have to look for the secondary molecular polymorphism of this products of amplification obtained from different accessions in order to be able to differentiate them on the basis of their genetic variability. That could be done by digestion with restriction enzymes, or other methods like DGGE, SSCP or even by sequencing.

The level at which different *Phytophthora infestans* accessions should be differentiated at molecular level, in order to consider that they belong to different genotypes,, possible different pathotypes, might be determined by correlating the molecular analysis with virulence analysis, reaction of accessions to different fungicides or mating type determination.

A synthesis of our results concerning analysis of genetic variability by molecular markers, in connection with mating type and susceptibility to metalaxyl of the *Phytophthora infestans* accessions, is presented in table 2.

As it can be seen the resistant or medium resistant *Phytophthora infestans* accessions were polymorphic either in respect of primarily polymorphism or in respect of secondary polymorphism. Eventually it could be said that different accessions were characterised by a specific molecular polymorphism.

Table 2. Molecular polymorphism, mating type and matalaxyl sensibilities on ten accessions of Phytophthora infestans from Romania

No.	Accession	Molecular polymorphism					Susceptibility to metalaxyl
		Primarily polymorphism:			CAPS secondarily polymorphism (haplotypes)	Mating type	
		OPC-09	OPC-20	ISSR-1			
1.	68	-	-	-	IIa	A1	Susceptible
2.	72	-	-	-	IIa	A1	Susceptible
3.	73	-	-	-	IIa	A1	Susceptible
4.	77	-	+	+	IIa	A1	Medium
5.	79	+	++	-	IIa	A1	Resistant
6.	83	-	-	-	IIa	A1	Susceptible
7.	88	+	+	+	IIa	A1	Medium
8.	89	-	-	-	IIa	A1	Susceptible
9.	91	-	+	-	Ia	A2	Medium
10.	98	-	-	-	IIa	A1	Susceptible

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Potato seed production

IN VITRO BASED POTATO SEED MULTIPLICATION SYSTEM COMPARING WITH CONVENTIONAL CLONAL SELECTION MODEL

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Main differences of these potato seed production models are the type of the initial material for starting multiplication and number of the repetition of the multiplication. In vitro based potato seed multiplication system obtains minitubers under controlled condition and continues open field production. So productivity, agronomical practices and economical point to continue multiplication differ from clonal selection model. Main reason to create the difference is the initial material characteristics.

When the potato seed production program, which is derived from mini tubers obtained through in vitro culture which is thought expensive as foundation stock material production system, is compared with the conventional potato clonal selection program, in considering of the seed potato tuber production even at basic level, quality and cost advantages of the minituber derivation is found higher.

Mini tuber derived potato seed multiplication has the advantages of reaching high profit level in low number of production cycle and low degeneration rate under the condition of low exposure of open air production.

Potato seed is being supplied to the farmers in three and at most four years of production cycle in mini tuber derived potato seed production scheme. In addition to the yield response affected by the high quality of potato seed derived from mini tubers, production cost that gives a high chance of competition to the mini tuber derived seed.

Clonal selection program even though the initial cycles might apply in somewhat controlled condition such as aphid proof net house, open field production cycles are definitely more than mini tuber derived program in number. This more cycle of open field exposure with many contamination risk factors in clonal selection type of potato seed production, makes mini tuber derived programs more secure.

Key Words: Potato, Seed, Minitubers, In vitro

GM POTATO AS A CHALLENGE FOR POTATO VIRUS PREVENTION – ECONOMIC ANALYSIS

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Introduction

In the last couple of years, the potato virus problems have increased explosively in Finland. (Kortemaa 2006). In Finland, the Y, A, S and M viruses spread by plant-lice cause the greatest harvest and quality losses, but the production areas in Finland and northern Europe are also infested with the mop top, which spreads with soil.

Most viruses are spread rapidly with plant-lice. The louse's probing sting on the leaf is sufficient for transmitting the virus to the louse, and another probe in another plant is sufficient for spreading the virus there (Valkonen 2006). The fact that plant-lice prefer not to remain in potato plants increases the spreading potential of the viruses. Most plant-louse species do not accept the potato as a source of nutrition.

The spreading of viruses transmitted by the proboscides is swift and unpredictable in the plant population. There is no time to stop its spreading by killing the plant-lice with pesticides. In fact, the pesticides may even have adverse effects. In some tests and in practical farming work, it has been tested whether spreading mineral oil on the potato population will slow down the spreading of viruses, but this has shown little effect (Tiilikkala 1993, Tiilikala and Kurppa 1988).

The worst sources of viruses are infected potatoes within the population. Infected seed potatoes and sprouts growing from the residual potatoes of the previous harvest are the worst. Plant-lice landing on these have a short distance for transmitting the virus to healthy specimens.

There are no prevention methods for potato viruses spread by insects apart from the use of virus-tolerant varieties and border strips for potato farming areas. Potato farming is centralised in Finland to such an extent that it is difficult and economically impossible to implement the border strips. In practice, the only means to fight the viruses is to further breed virus-tolerant varieties. Although the Research Group on Applied Plant Biotechnology at the University of Helsinki has studied the viral tolerance of the potato since 1990 and patented a potato species modified with the P1 gene tolerant of potato virus Y (PVY, genus *Potyvirus*), a virus-tolerant potato is yet to emerge on the commercial market. Legislation and permit procedures concerning the deployment of GM varieties are not ready yet, and consumer resistance to GM varieties is firm.

This study has been restricted to studying the cost of virus risks in seed potato production as well as to estimates of how these costs can be lowered with the implementation of genetic engineering. Furthermore, the research evaluates the distribution of cost and benefits of genetic engineering in the supply chain of the potato.

Research data and methods

The research data of this study has been collected from seed potato inspection statistics of the Finnish Food Safety Authority (Evira). Evira inspects seed potatoes with laboratory tests for viruses. Potato viruses in Finland have previously not caused significant costs and the risks from viruses have been small. In 1998–2003, the Plant Production Inspection Centre inspected 2,235 seed potato lots for viruses. Of these, five lots (0.2%) were rejected due to the A and Y viruses, and in 61 lots (2.7%) the seed potato was degraded to a lower seed grade.

During the last two years, the situation has rapidly changed. In 2005, a total of seven lots (2.7%) were rejected for virus content and 49 lots (19.2%) were downgraded to a lower seed class. In 2006, the virus situation was even worse: more than half of the tested potatoes had the virus, of which 85% was the Y virus and the rest the A virus. 10 seed potato crops were rejected because of the virus, totalling approximately 56 hectares (3.2%), and the seed class has been lowered on every third plantation.

The lowering of the seed potato classes and rejection of lots as unsuitable for seed potato may cause great economic loss at the farm level and also cause great welfare losses at the societal level.

Economic impact caused by viruses and the distribution of the impact is estimated based on farm model calculations that relies on agricultural profitability bookkeeping data and previously executed profitability surveys (e.g., Tuomisto 2007). The distribution of benefits and costs within the supply chain was estimated based on the previously conducted contract production research (Tuomisto 2007).

The farm model calculations are based on farm-specific net profit calculations, which were carried out for average-size seed potato farms of 15 hectares. The calculations are used for estimating the distribution of costs caused by viruses and the benefits of eliminating virus risks across the various parties.

The profitability of seed potato production was inspected with net profit calculations and profitability coefficients:

$$\pi_{AVERAGE} = \frac{\sum_{t=1}^{T}\left(\sum_{i=1}^{n} p_i y_i - \sum_{j=1}^{m} w_j x_j + \sum_{k=1}^{z} s_k\right)}{T} \qquad (1)$$

The average net profit $\boldsymbol{\pi}$ is an average subtraction between sum of total returns and sum of total costs plus sum of total support.

The seed cost of a seed potato and food potato producers cannot only be measured by the price of seed potato, as the various seed potato producers offering seed potato produce different sizes of seed. The seed cost of a food potato hectare is a better indicator of benefit of a food potato producer. The seed cost of a food potato hectare can be calculated by the following formula:

$$C_{Ri} = \frac{\sum_{t=1}^{T} \sum_{i=1}^{n} k_{it} z_i \frac{10^5}{\alpha_i \beta_i} w_{it}}{T} \qquad (3)$$

Thus, the average seed cost of a potato hectare, $\boldsymbol{C_{Ri}}$ (€/ha), has been calculated by multiplying the required seed quantity (pcs/ha), obtained from the planting distance $\boldsymbol{\alpha_i}$ (cm) and spacing $\boldsymbol{\beta_i}$ (cm) of each variety and seed size, with the seed weight $\boldsymbol{z_i}$ (g/unit) and by multiplying the result thus obtained with the average deflated seed sale price $\boldsymbol{w_{it}}$ (cents/kg) of 2000, considering the share of seed produced in the different years, $\boldsymbol{k_{it}}$ ($\boldsymbol{k}$=0,…,1, i.e., $\boldsymbol{\Sigma k}$=1) in the various seed size classes ($\boldsymbol{i}$=1…n) and by dividing the sums hence obtained with the number $\boldsymbol{T}$ of years ($\boldsymbol{t}$=1,…,m) inspected.

In Finland, seed potato can be increased approximately 4.5 times with cloned seed potato, first pre-base-seed class, before it is sold as seed potato to the food potato producer. Te production chain could also be longer, up to seven steps. Viruses are one factor that shorten the production chain of seed potato and reduce the clonability of seed potato.

In this study, production calculations have been used for estimating cost of production of each seed potato class. Finally, the cost of production has been added up. In Formula 4, the sum $\boldsymbol{V}$ indicates the value of the entire seed potato production chain of Finland. In the formula, ($\boldsymbol{i}$=1…n) represents the hectare-specific production cost of each seed potato class, ($\boldsymbol{j}$=1…m) adds up the hectares farmed in each seed class and ($\boldsymbol{h}$=1…l) adds up the total cost of all seed classes.

$$V = \sum_{h=1}^{l} \sum_{j=1}^{m} \sum_{i=1}^{n} w_{hij} x_{hij} \qquad (4)$$

The cost of production per produced seed potato kilogram is reduced as the production chain is prolonged.

Results

The production cost of seed potato production in Finland is an average 47.80 ¢/kg. In 1998–2003 the virus risks have increased the cost of production of seed potato by an average of 1.7%.

During the last two years, the situation has rapidly changed. In 2005, the virus risks increased the cost of seed potato production by 13.3%. In 2006, the virus situation was even worse: the virus risks increased the cost of seed potato production by 17.8%.

A GM variety that can reduce the long-term problems of potato viruses would decrease the cost of production of seed potato. During the review period of 1998–2003, the risks of viruses spread by plant-lice increased the cost of production of seed potato by an average of €226/ha. In other words, if it were possible to completely eliminate the virus risks with a GM variety, it could reduce the cost of production of seed potato by € 226/hectare and increase the profitability of seed potato production by the same amount if the beneficiary was the seed potato producer alone. If the beneficiary was the food potato producer, the seed cost would lower by € 34.49 per planted food potato hectare. If the beneficiary was the breeder of the seed potato, it could collect an additional 1.45 ¢/kg of variety royalty, and if the beneficiary was the variety representative, the price of the base seed could be € 226 higher per planted seed potato hectare. In other words, the price of the base seed potato could be approximately 6 ¢/kg higher.

It is difficult as yet to determine if the harvest years 2005 and 2006 are statistical deviations (outliers) or whether the virus problem has come here to stay. If we inspect the cost incurred by virus risks as an average of the years 1998–2006, the production cost is increasing, while the benefits from reduced virus risks with GM varieties increase considerably higher, up to €644 per seed potato hectare. If the beneficiary was the seed potato buyer, the food potato producer, the seed cost would lover by € 97.97 per planted food potato hectare. If the beneficiary was the breeder of the seed potato, it could collect an additional 3.13 ¢/kg of variety royalty, and if the beneficiary was the variety representative, the price of the base seed could be € 644 higher per planted seed potato hectare. In other words, the price of the base seed potato could be approximately 16.9 ¢/kg higher. The attribution of benefits to the various parties depends on the production agreement and market position of each company producing seed potato or food potato (Tuomisto 2007, Tuomisto 2004).

The calculation above inspected the cost of virus risks at the farm level. A virus risks cause societal costs in period 1998-2006 an average by 837,200 euros per year. In addition to this, the virus risks also shorten the production chain of the seed potato. In an optimal situation where no virus risks exist, the supply chain of the seed potato from the first class of the pre-base-seed class, the clone (class SS), can be up to seven steps long before the seed potato is sold as seed to the food potato producer. In this scenario, an area of 0.013 hectares of first-class pre-base seed would be sufficient after seven seed potato generations to produce the base seeds of 1,500 seed potato hectares, which is enough for all of Finland. In 1998–2003, a potato virus has been the cause of seed potato class lowering below the objective in one third of the cases in average. During a review period of 1998–2006, a potato virus has been the cause in more than 50% of the cases where the seed potato class has lowered below the objective. This has strongly shortened the production chain of the seed potato. The production chain of the seed potato in Finland has an average of 4.5 steps. Figure 1 presents the increase of seed potato production cost caused by the shortened supply chain (€/kg) and the impact of the increased cost on the entire society per harvest year.

The cost of production of seed potato in a five-step production chain is approximately ¢46.48/kg. At the same time, the total cost of production of the entire Finnish seed potato chain is €13.8 million. If the seed potato chain were to shorten to four steps, the cost of production of seed potato would increase to ¢49.12 while the total cost would rise to €14.5 million. The additional expense for the entire production chain would then be €780,109.

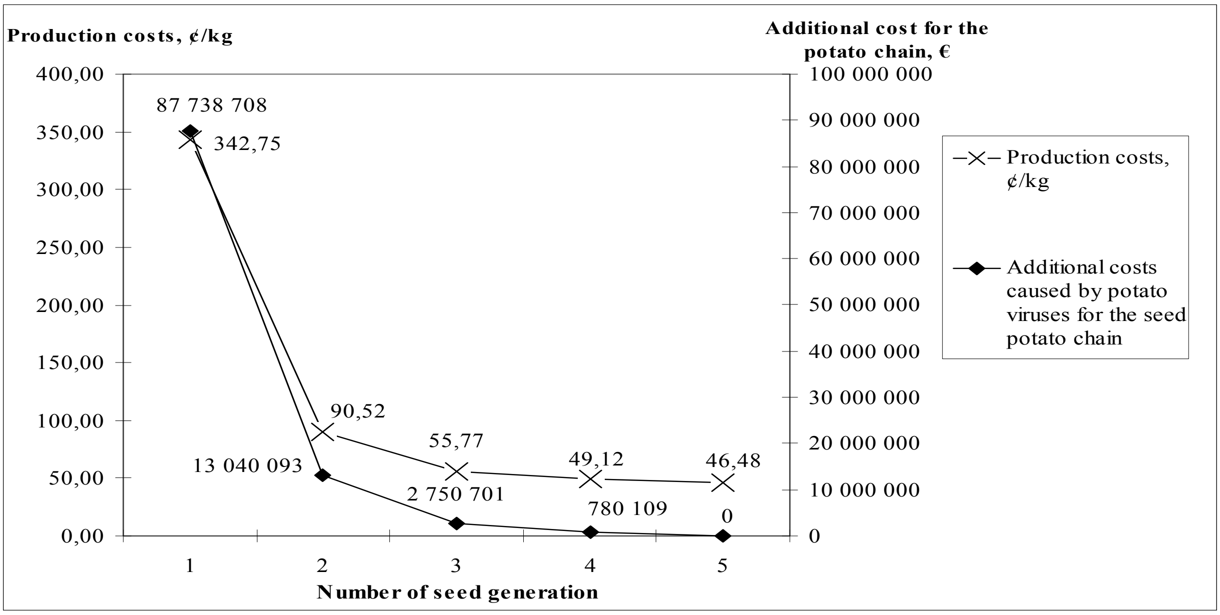

Figure 1. The impact of the number of seed potato generations on the cost of production of the seed potato, and the total cost of additional expenses caused by potato viruses for the entire potato chain.

Conclusion

Potato viruses have become a problem in Finnish seed potato production in the recent years. Potato production is located in a narrow area on the western coastline of Finland, and potato farms are located close to each other (Tuomisto and Huitu 2006). Furthermore, the production of seed potatoes and food potatoes is primarily concentrated in the same areas. Therefore, viruses spread by plant-lice have unobstructed paths of transmission from population to population. With the arrival of the virus problem in Finland, it is difficult to entirely eliminate it.

There are no other efficient protection methods against potato viruses spread by plant-lice than refining the potato plants for virus resistance. Due to the ease and high speed of transferring a virus resistance gene, the GM potato can be seen as the only realistic way of attaining virus-resistant varieties, in lowering virus risks and subsequently in lowering the net loss to the society.

Although the Research Group on Applied Plant Biotechnology at the University of Helsinki has studied the viral tolerance of the potato since 1990 and patented a potato varieties modified with the P1 gene tolerant of potato virus Y (PVY, genus *Potyvirus*), a virus-tolerant potato is yet to emerge on the commercial market. Legislation and permit procedures concerning the deployment of GM varieties are not ready yet, and consumer resistance to GM varieties is firm.

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STUDIES ON DEVELOPMENT OF NATIONAL POTATO SEED PRODUCTION SYSTEM IN TURKEY

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INTRODUCTION

Potato is one of the most important agricultural crops with approximately 5 million tones production on 160 000 ha planting area annually, in Turkey. Annual potato seed demand is around 450000 tones in the country. However, there is no regular seed potato production system in the country and usage of certified potato seed accounts only 10% of the total annual demand of the country. The potato cultivars, which were bred in Europe or North America, are used in the country, and seed supply system depends on multiplication of imported seed. A project was started to establish a "National Potato Seed Production System" with the cooperation of research institutes, universities and private sector under coordination of the Ministry of Agriculture and Rural Affairs in 2005.

The project has consisted of three sub-projects: (1) Determination of the most suitable seed potato production areas, (2) Development of the basic seed production programs, (3) Development of potato cultivar breeding programs. Each subproject also consists of several work packages. The aim of the project is not definitely close the country to enter of foreign cultivars or seed tubers, but it is aimed to enlarge the usage of certified seed in potato production. The project will be conducted as three-year terms and the first term will terminate at the end of 2008. In the first term, we planned to collect fundamental knowledge about characteristics of possible seed areas and to build basic infrastructures for basic seed production and cultivar breeding. In this presentation, we will give the brief evaluation of the first term of the project.

DETERMINATION OF SEED POTATO PRODUCTION AREAS

Identification of suitable seed production areas is very important for healthy and economic seed potato production as well as sustainability of production. There is no special area, where devoted to seed potato production in Turkey. Seed and ware potatoes have been produced in the same areas for years. This entails difficulties in application of phytosanitary precautions for seed potato production. Turkey faced a serious wart problem in last decade in some areas where majority of seed potatoes are produced. Then both ware and seed producers searched new potato production in different part of the country and they have shifted their production into these new areas. Such an uncontrolled expansion of potato production areas impend the sustainability of potato production in the country. Hence, identification and registration of seed potato production regions in the country seems as the prerequisite for successful and sustainable seed potato production in Turkey.

Although seed and ware potatoes have been produced within same regions, some regions are stand out with better performance of their seeds. These areas are high plateaus, which are characterized with high altitude (>1000 m) and cool day/night temperatures during summer period. There also some regions having similar altitude and climatic conditions, but no potato production history in the country. Hence we determined some potential seed production areas according to their soil and climatic conditions. We also considered some other criteria such as distance of potential areas to common potato production regions, size of agricultural lands, irrigation facilities etc., to determine the potential seed areas. Thus we included fifteen provinces in total to the project to evaluate their potential as seed potato area. Then, following studies have been conducted in these areas:

- Existence of major fungal, bacteriological and viral diseases of potato,
- Existence and population dynamics of major insect pests of potato,
- Existence and population growth of virus transmit aphid species,
- Determination of the most suitable planting and haulm killing dates for each province,
- Determination of seed degeneration rate in each province,
- Comparison of growth and yield performances of potato seeds, which were grown in evaluated sites, in common ware potato production regions.

All above work packages were started in 2006. After two years study, no significant problem threaten the seed potato production was found in twelve of fifteen sites. The studies on identification of seed potato production areas will terminate at the end of 2008. At the end of 2008, the most suitable seed production areas will be determined and will be submitted to the Ministry of Agriculture and Rural Affairs to register them as "Seed Potato production Area"of Turkey.

DEVELOPMENT OF THE BASIC SEED PRODUCTION PROGRAMS

Currently certified seed potato production ground on multiplication of imported basic seed in Turkey. The super elite or elite potato seed tubers should be produced within the country to build a National Seed Production System. Then, a systematic seed multiplication scheme from super elite to certified seed should also be organized. Direct usage of mini tubers in production of certified seed have also been increased in recent years in the world. There are a few private enterprises, which produce mini tubers, in Turkey. However, their capacity not enough to met country's seed demand at this moment.

In our project, four pilot institutions were established for basic seed production. These institutions are responsible for production of virus free plantlets and mini tubers via meristem culture and greenhouse production. Then, they will distribute mini tubers to the other institutions for further multiplication until certified seed category. The seed multiplication will be done in evaluated seed areas. Hence we can determine the multiplication and degeneration rate of seeds in each potential seed area. This also allows us the observation of the potential problems which can be faced during seed multiplication.

The Project is not aimed to meet basic seed demand of the Turkey by pilot institutions. The main objective is establishing a logic model for basic seed production in Turkey. The main infrastructure of four institutions completed in 2006, and they produced the first mini tubers in greenhouse at the beginning of 2007. Then mini tubers were planted into net house for the first generation multiplication during 2007 summer. We are planning to increase the minituber production capacity in 2008.

DEVELOPMENT OF POTATO CULTIVAR BREEDING PROGRAMS

The efforts on potato cultivar breeding very limited in Turkey up to now. There is only one cultivar (Nif) bred in Turkey via crossing. The cultivar Nif entered to the National Cultivar List in 1998, but this variety never found acreage in commercial scale. European or North American potato cultivars are grown in Turkey. It is expected that the cultivars bred in a certain environment can show better adaptation to this environment than introduced ones. Furthermore, having intellectual property rights of cultivar is one of prerequisites of establishing a seed production program. Therefore it is very important to start a cultivar breeding program simultaneously with studies on development of seed production program.

The sub project on cultivar breeding program was started with planting of parents into greenhouses at two institutes in March 2007. We select commonly grown ware and processing potato cultivars in Turkey as parents for the first year crossing. The hybridization studies were conducted between May and July. There were fifteen crossing combination and we harvested around 100000 hybrids true potato seeds in August. The hybrid true potato seeds (12000) were sown into greenhouse in Mediterranean coastal region in November 2007. Then the first generation hybrid tubers were harvested in March 2008. Hence we saved one year in breeding program with using winter season. We didn't do any selection at the first generation. Four tuber families were created from each hybrid genotype. Each tuber family was send to different institutions in for further evaluation in field generations during main crop season. The hybrid tubers were planted in June at all institutions for the first generation selection. The breeding program will continues as a pipe-line program and we are planning to broaden the program with special breeding aims. We also are planning to include wild species to the program in the next term of the project.

CONCLUSION

The development of a potato seed production and cultivar breeding program is an urgent necessity for Turkey. This project is a first step to alleviate this necessity in some extent. However, it is obvious that it is impossible to obtain miraculous results for overcome all the demands with one project. There is also shortage of knowledge and qualified staff for such a program in the country. The project will also contribute to these aspects since around sixty researchers from eleven institutions are currently employed in the project. Moreover, we are in cooperation with ten private enterprises dealing with seed potato production in Turkey. Consequently, we can realize significant progress in seed potato production and cultivar development if the project successfully continues.

THE SPROUT/SEED-POTATO TECHNOLOGY: A REVIEW ON THE INNOVATIVE IDEA OF EXPORTING/ IMPORTING SPROUTS FOR SANITARY MOVEMENT OF VIRUS-FREE "SEED-POTATO" STOCKS

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Introduction

Traditionally, the seed-potato (*Solanum tuberosum* L.) production and certification program in Brazil has been dependent on imported virus-free seed-tubers. A record of constant declining in Brazil's dependence on imported seed-potato stocks has been pointed out : from 15,000 ton in the 80's; around 8,000 in the 90's, to near 3,000 ton over the past 5 years (Andreatta, 2006). Economic and sanitary factors, such as high cost of imported stocks and the possible introduction of tuber-borne pathogens, has initiated local production of minituber, mostly from tissue-cultured plantlets.

Sprout/seed-potatoes

Although sprouts are identified in many textbooks as a propagating material, it has been described as a laboring and thus, discouraging system: starts with sprout tip cuttings; rooting and transplanting to: 1- pots, for further stem cutting, leaf-bud cuttings, or (2) soil, for producing normal tuber (yielding 0,5 to 1,0 kg/plant), thus indicated for areas where labour is available and cheap (Bryan et al., 1981; Struik and Wiersema, 1999), like in Argentine, where sprouts are used for tuber production by small subsistence farmers (Huarte, 2005). Other authors not even mention sprouts as a propagating material when reviewing on seed-potato new technologies or improvement programs (Slack, 1993; Assis, 1999; Stark and Love, 2003).

Since our first report demonstrating successfully that it is possible to obtain a 2-fold average increase on the number of virus-free seed-tuber stock by just planting detached sprouts in pots containing potting mixes (Souza-Dias & Costa, 1985), it took about 10-years to start transferring this technology to large as well as small farmers in Brazil (Souza-Dias & Costa, 1998). Since beginning the 90's the hundreds of thousands of sprouts, which are (used to be) routinely detached and discarded from virus-free, high-grade tuber/seed-potato stocks in Brazil have been used and recognize as a largely available propagating material (seed-potato), ease-to-have; ease-to-handle; ease-to-plant, for a simple and inexpensive, large-scale, minituber/seed-potato production, on a direct use system (Gallo,2007).

Since the mid 1990's, millions of virus-free sprouts, detached from seed-potato tubers, which used to be discarded as garbage, i.e, treated as "trash", have become a valuable additional source of "Pre-Basic" seed-potato stock. This shift is the result of the innovative sprout/seed-potato technology which has been successfully adopted by traditional seed-potato producers, and particularly by small farmers (greenhouse horti-fruit producers and citrus nursery growers). As not depending on laboratory to produce, the sprout/seed-potato has been a feasible system of producing hundreds of thousands of virus-free minituber/seed-potato stocks. (Souza-Dias & Costa, 1998; Souza-Dias et al, 2001).

About 10 yeas ago, small farmers in Brazil began producing minitubers from sprouts supplied (initially for free) by large seed-potato farmers (Souza-Dias & Costa, 1998) as a simple, inexpensive method to mass-produce high quality seed-potatoes (Gallo, 2007). Traditional seed-potato growers, who routinely de-sprout their seed tubers prior to planting, have been contracting small farmers to produce minitubers from their own virus-free sprouts. Hundreds of thousands of high-grade virus-free minitubers have been produced using this technology. Farmers have confirmed that the sprout/seed-potato minituber system is a viable new source of income. Some small farmers who previously could not afford to purchase high grade, virus-free seed potatoes have become producers of minituber/seed-potatoes themselves. Although still a small part of the seed-potato production system in Brazil, over the past 5 years adoption of this technology has been increasing.

From the common and recommended practice of removing the apical sprout to enhance sprouting of seed tuber stocks before planting (Filgueira, 1999; Beukema & Van der Zaag. 1979), millions of sprouts (propagating material) are easily generated. The sprouts can be removed either manually or mechanically, e.g. when passing tubers through grading rollers before planting. Healthy, true-to-type sprouts can be harvested in this way with little or no additional cost. As a by-product of tuber/seed-potato stocks, sprouts are now recognized as healthy propagating material.. When compared to tissue cultured plantlets, the sprouts detached from seed-potato tubers carry some important advantages, including lower production costs and a high genotype fidelity, i.e., lower probability of somatic clonal variations (Hayashi, 2007; Ranalli, 1997). In Brazil, virus-free minitubers from laboratory tissue culture plantlets have an average sale price of R$ 0,32 – 0,35 (US$ 0.18 – 0.20) per unit, which is near 65% more expensive than minitubers from the sprout/seed-potato technology: R$ 0,18 – 0,23 (US$ 0,11 – 0.12).

Indeed, it seems hard to believe that an apparently obvious method of producing hundreds of thousands pathogen-free minituber/seed-potato stocks had been ignored as a method of mass production of minituber/seed-potato. This ignorance explains why sprouts from high grade seed lots continue to be discarded in countries that could use this technique the most. The potential of the sprout/seed-potato technology has been recognized internationally by potato specialists as a new seed-potato production system to be exploited. (Guenthner, 2006) It was one of nine "innovative technologies" nominated by an international jury for the "Innovation Award" at the Potato World Congress held in Emmeloord, The Netherlands, in September, 2005, organized by the Agricultural Promotion Projects of the Chamber of Commerce Lelystad 39051673 (www.potato2005.com).

Regulatory and import issues

Increasing adoption of the sprout/seed-potato technology necessitated official regulation for certification of the minitubers. Concerns included traceability of sprout origin, phytosanitary inspection procedures and royalties for varieties having Breeders' Rights protections. In an attempt to cope with the restrictions pointed out by seed-potato certification agents we have evaluated and introduced with success the innovative idea of importing only sprouts as high-grade, seed-potato stock (Souza-Dias et al, 2007) New export-import seed-potato protocols which respect all applicable breeder and/or variety property rights are being developed (Francelino, 2005), and sprouts are now being virus tested via ELISA immunoassay in the same manner as for tuber/seed-potato lots (Giusto, 2006). Therefore, just like the conventional importation of tuber, sprouts can also serve as seed-potato stocks to supply local demand for annual renewal of virus-free seed-potato stocks. The idea is now gaining interest among potato producers in other countries (Souza-Dias, et al., 2007; Gallo, 2007)

During a 4-year of USA (2003-2006) and 1-year Canada (2007), export/import testing sprouts "as seed-potato", carrying official phytosanitary and varietal certifications of the sprouted tubers, have been successful: phytosanitary and genetic fidelity results, production of minitubers from sprouts and tuber/seed-potato lots from minitubers have all been excellent. Shipments of 5 to 30 kg of sprouts (20-40 cm long, 2-5g each) produced 1.8 to 3 minitubers (2-4 cm) from whole sprouts or 2-node sections. Recognizing the scientific advantages of the new sprout/seed-potato technology, the Brazilian Ministry of Agriculture/ Department of Sanitary Inspection is encouraging the initiation of commercial imports of potato sprouts. Currently, a Brazil-Canada evaluation of the economics of a potential export-import market for the sprouts is being investigating. However, to develop an international market for potato sprouts, some issues must be resolved, as listed below:

1. A fair unit price for sprouts, which is competitive with the existing propagating material. A preliminary price of US$ 0.06 has been offered, whereas tissue cultured plantlets cost about US $ 0.30 in Brazil. Clearly there is a broad gap. Production costs in potential exporting countries have not yet been established.

2. Moderate transportation costs. Trial shipments of sprouts via courier were less than 7 days' duration, but cost US$10 to $40 per kg. Alternatively, post office express mail was much less expensive but the delivery time exceeded 34 days. Happily, despite the long transit under unknown conditions, 60-80% of the sprouts germinated and produced normal minitubers. Sprouts appear to be a rather durable product!

3. Mechanisms to respect Breeders' Rights and to identify lot number, variety, producer, phytosanitary and other data from the seed-potatoes used to produce the sprouts, for certificates of origin and traceability.

4. The marketability of the de-sprouted *vs.* regular seed-tubers.

5. Costs to initiate, remove and package the sprouts.

The sprout/seed-potato, as compared to the conventional whole tuber/seed-potato, is a less expensive, more easily transported and more phytosanitary method for the safe movement of propagating material. That aspect seems a clear and undoubted commercial advantage. Compared with the importation of the conventional whole tuber/seed-potato, a country importing only sprouts, would run less risk of importing tuber borne problems.

Toward development of an international market for potato sprouts, resolution to the issues listed above is the next step to full development of this innovative technology for the benefit of the potato industry in Brazil and elsewhere. As potato producers everywhere face increasing pressures to remain competitive, the sprout/seed-potato technology is a new way to improve profitability. In addition, facing also a business competition for agricultural land with potential more valuable crops such as those for energy supply, we think the sprout/seed-potato is one "real" alternative.

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Agronomy

CLIMATE CHANGE AND VOLUNTEER POTATOES IN SLOVENIA

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INTRODUCTION

Climate changes caused by global warming already influence the potato production in Slovenia. Extremely hot and dry weather during summer often causes physiological disorders such as re-growth and secondary tuber formation which result in a high number of small tubers that are left in the soil after harvest. If they do not freeze during the winter which follows, they can cause serious problems in the following crop or even later in the rotation.

MATERIALS AND METHODS

The work consisted of two parts: monitoring of farmers' fields a year after potato crop and setting up of micro trials.

In the first part volunteer potatoes were monitored in the crops that followed potatoes. Five different fields located in the central part of Slovenia were selected in autumn 2006 and 10 plots (1 m^2 each) per field were monitored in May 2007. Each plot was selected by chance. The Slovenian variety Pšata was grown on four chosen fields and the variety Romano on one field. Pšata was selected because of high tuber set. The emerged stems were counted on each plot in May.

Micro trials were set up on three different locations in November 2006. Two of them were located in the central part of Slovenia near Ljubljana with continental climate characteristic for its rather severe winters (Figure 1). Two different soil types were chosen. One trial was established in the coastal part with Mediterranean climate characterised by mild winters on deep red soil called "terra rossa" (Figure 2). Air and soil temperatures were measured during the experiments. Complete randomized block design with four repetitions and two factors (tuber size and planting depth) was used. Tubers were planted at the depths of 0-5 cm, 10-15 cm and 20-25 cm. Two different grades of seed potatoes of the variety Sante were used: tubers smaller than 25 mm in diameter and tubers larger than 45 mm. Twenty tubers were planted per plot of 1.2 m^2 with planting density of 16.7 plants per m^2. The emerged stems were counted on each plot in May 2007 and the tubers were harvested and counted in June.

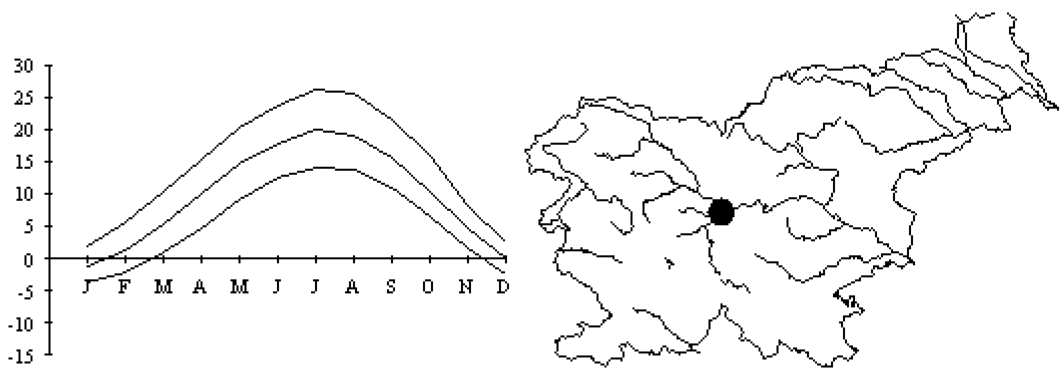

Figure 1: Average daily temperatures in Ljubljana

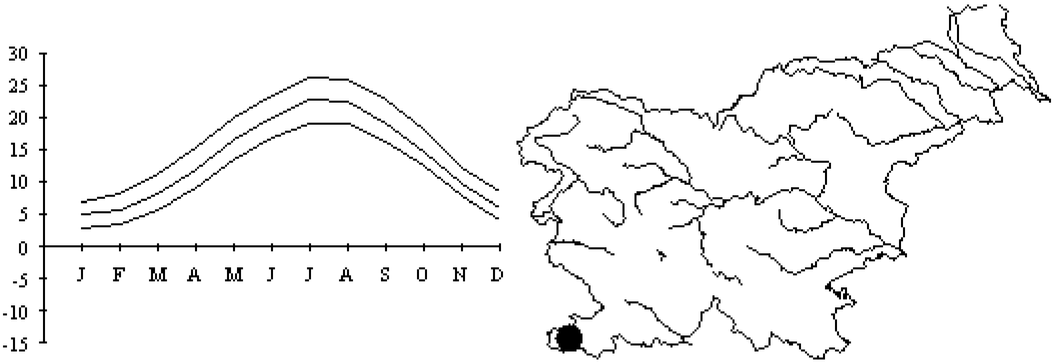

Figure 2: Average daily temperatures in Portorož

The work started in the season 2006/2007 and will continue in the season 2007/2008.

RESULTS AND DISCUSSION

Field monitoring of the appearance of volunteers resulted in very high number of volunteer plants with the variety Pšata on all fields regardless of the following crop. Considerably less stems emerged with the variety Romano. The highest stem number was found in the field 2 and it was 56 stems per plot, which is more than the recommended stem density of any potato crop. There was not any plot with no stems emerged. The average number of the emerged stems was between 3.1 and 24.5, which is extremely high and caused huge problems to the farmer. The high coefficient of variation indicates very high variation among the selected plots (Table 1).

Table 1: The number of emerged stems in five selected fields situated in the central Slovenia in May 2007

	Shallow sandy soil		Deep loamy soil		
	Field 1	Field 2	Field 3	Field 4	Field 5
Variety	Pšata	Pšata	Pšata	Pšata	Romano
Crop	Maize	Beans	Onion	Maize	Maize
Average	13	24.7	16.5	18.2	3.1
Maximum	29	56	26	34	5
Minimum	3	11	9	8	1
KV %	60.1	58.9	42.2	49.2	

From Table 2 we can conclude that most of the planted tubers survived the winter 2006/2007. That was not true for the tubers which were planted below the surface and were almost completely frozen in the continental climate. The emergence was better in the treatment with large tubers at planting depth of 10-15 cm compared to 20-25 cm. This indicates the influence of other factors beside the freezing soil temperatures which affect the emergence of volunteer plants. More tubers survived the winter on sandy soil than on loamy soil.

Table 2: The percentage of emerged plants in micro trials on three locations in 2007

	Central Slovenia				Seaside	
	Shallow sandy soil		Deep loamy soil		Deep terra rossa soil	
Planting depth	Large tubers	Small tubers	Large tubers	Small tubers	Large tubers	Small tubers
0 - 5 cm	1.25	0.00	0.00	1.25	73.75	65.00
10 - 15 cm	95.00	76.25	81.25	61.25	95.00	96.25
20 - 25 cm	87.50	72.50	68.75	33.75	93.75	68.75

The emergence was even higher in the non freezing conditions of the seaside. It was more than 65 % in all three depths and with both tuber sizes. There were no differences among tuber sizes at the depth of 10-15 cm with small tubers performing even slightly better.

The climate is changing. From Table 3 we can see that the temperatures are getting higher also in Slovenia. The period average of yearly sum of average daily temperatures above the threshold of 18 degrees Celsius is higher by 192 degrees comparing the periods 2001-2006 and 1971-1980 in Ljubljana. The same difference is 169.5 degrees in Portorož. We can conclude that summers in the continental Ljubljana in the last 6 years are warmer than the summers between 1971 and 1980 in the Mediterranean Portorož.

Table 3: The period average of yearly sum of average daily temperatures above the threshold of 18 degrees for four different periods in the continental climate of Ljubljana and in the Mediterranean climate of Portorož

Weather Station	Period				Difference between 1st and 4th period
	1971-1980	1981-1990	1991-2000	2001-2006	
Ljubljana	162.5	214.2	296.1	354.5	192
Portorož	323.3	374.7	440.6	492.8	169.5

(Source: http://www.arso.gov.si/vreme/podnebje)

We believe that on the basis of summer temperatures similar conclusions can be drawn for winter temperatures which have become milder. Consequently, they indicate bigger problems with volunteers in future in the case of mild winters due to further climate changes.

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STUDY THE POTATO - WEED INTERACTIONS

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Abstract

To study the competitive effects of redroot pigweed (*Amaranthus retroflexus*) and lambsquarter (*Chenopodium album*) on potato, an additive experiment was conducted in the spring of 2004 and 2005 in split-split plot based on randomized complete block design with 4 replications at the Seed Potato Production Station of RAN in Firouzkooh. Treatments were included 2 weed species in main plots (*Amaranthus retroflexus* and *Chenopodium album*), weed density in sub plots (2, 4 and 8 plants per meter of row) and relative time of weed emergence in sub-sub plots (8 and 4 days prior to potato and the same time with potato emergence in 2004 and the same time with potato, 2 and 4 weeks after potato in 2005). Results showed that both weed species reduced biological yield, height, LAI, CGR and RGR. TDM reduction in 2005 were 27.5 and 25.1 percent less than 2004 in response to lambsquarter and redroot pigweed. Increasing weed density resulted in reduction in potato LAI, but leaf area distribution in canopy layers was not affected and maximum LAD (Leaf Area Density) was recorded in second layer (20-40 cm) in all treatments. In 2004, redroot pigweed in 2, 4 and 8 plants per meter of row put 29, 42.1 and 44.6 percent of LAI above the potato canopy, respectively. These amounts for lambsquarter were 30.6, 38 and 43 percent. By this mechanism, redroot pigweed and lambsquarter intercepted 54.8 and 53.3 percent PAR above the potato canopy in 2005 and 39.6 and 34.9 percent in 2005, respectively.By inceasing weeds interference, light interception of potato was reduced. So, in 8 plant of lambsquarter per meter of row , potato light interception was reduced 43.3 and 53.5 percent in 2004 and 2005. In third level of redroot pigweed (8 plants per meter of row) reduction of potato light interception was 56.5 qnd 66.8 percent in 2004 and 2005, respectively. On average, redroot pigweed caused 49.6 percent more potato yield reduction in comparisn with lambsquarter.

Keywords: potato, redroot pigweed, lambsquarter, canopy structure, competition.

Introduction

The use and application of herbicides was one of the main factors enabling intensification of agriculture in developed countries in past decades. However, increasing herbicide resistance in weeds, the necessity to reduce the cost of inputs and the widespread concern about environmental side effects of herbicides have resulted in greater pressure on farmers to reduce the use of herbicides. This has led to the development of strategies for integrated weed management (Kropff & Lotz, 1992). A successful integrated weed management program for the control of weeds cannot be implementing without a clear understanding of inter-specific competition between major field crops and weeds (Eslami *et al.,* 2006).

Weed density and relative time of weed emergence have impacts on crop weed interactions. The timing of weed emergence relative to crop emergence is important to crop growth and yield. Weeds emerging before the crop cause greater yield loss (Bosnic & Swanton, 1997; Dieleman *et al*., 1995; Knezevic *et al*., 1994 and 1995; Murphy *et al.,* 1996; O'Donovan *et al*., 1985; Steckel & Sprague, 2004; Chikoye *et al*., 1995; Kropff 1988), produce more seed (Bosnic & Swanton, 1997; Peters & Wilson, 1983), and have higher shoot weights and competitive indices (Martin & Field, 1988).

Potato (*Solanum tuberosum* L. *var*. Agria) is one of the most important field crops in Iran and due its role in providing food and proteins for an increasing population; area under cultivation and yield of potato is increasing rapidly. In 2005, the area under cultivation of potato was about 170000 hectare and its yield was 25 tons per hectare (Anonymous, 2005). Redroot pigweed (*Amaranthus retroflexux*) and lambsquarter (*Chenopodium album*) are highly competitive weeds that are widely distributed through cropping area of Iran and cause large potato yield losses. There is limited research on competition between these weeds and potato and there are a few references to study the effects of various weed density and relative time of emergence on potato. The weediness of these species has been attributed to their strong competitive ability with crops, flexible germination requirements, high reproductive capacity (VanGessel & Renner, 1990; Knezevic *et al*., 1997; Murphy *et al*., 1996; Guo & AL-Khatib, 2003).

Ivany (1986) reported reductions in potato tuber yield with increasing in duration of quackgrass (*Elytrigia repens*) interference. Nelson & Thoreson (1981) found that potato tuber yield was reduced to a greater extent when weeds emerged prior to the crop than when the weeds emerged with of following the potato. They indicated that when weeds emerged early in the potato field, yield reduction was 54% compared to a reduction of 16% in comparable plots with late weeds emergence. Tuber yield were 31% and 39% less in the low (58 plant/m2) and high weed (311 plant/m2) density plots. Competition between foxtail and potato for 2 weeks after potato planting caused 19% and 29% reduction in total and marketable tuber yield. But after 10 weeks interference, these values increased to 69% and 86%, respectively (Wall & Friesen, 1990). Love et al. (1995) showed that emerging weed before potato reduce yield 68%, but when weeds emerge after potato, this reduction will be 25%.

Models of weed-crop competition should be an essential part of cost-effective decisions in weed management. In spite of the growing importance of redroot pigweed and lambsquarter competence on potato, there are not models to establish quantitative information about the influence of these weeds on potato yield.

The hyperbolic yield-density model for the description of yield loss in relation to weed density is the most widely used regression model to describe effects of competition at a certain moment (Cousens, 1985b; Spitters *et al.,* 1989). This simple empirical model usually gives satisfactory post hoc descriptions of crop loss but tend to fail when it is used predictively because its parameters are highly variable between experiments (Kropff *et al*., 1984; Firbank *et al*., 1990). Also, the nonlinear hyperbolic equation (Cousens *et al*., 1987) has been widely used to model the effects of relative time of weed emergence on crop yield. However, the model assumes that the maximum percentage yield loss does not change with the relative time of weed emergence (Fu & Ashley, 2006).

The objective of this study was to model the effect of density and relative time of weed emergence of redroot pigweed and lambsquarter on potato yield in order to provide a decision tool for the Iranian's farmers.

Materials and Methods

Field experiments were conducted at the research station of seed Potato Production of RAN in Firouzkooh (33º 55' N, 52º 50' E and 1975 m mean sea level) in 2004 and 2005.

The soil of the experiment plots was silt loamy in texture with %sand, %clay and %silt; pH=7.6; EC=1.23 mmose/cm; organic carbon percentage=0.5%; absorbable phosphorus = 20 ppm; absorbable potassium = 349 ppm and total Nitrogen = 0.05%

This experiment was done in split-split plot based on randomized complete block design with 4 replications. Individual plots size was 3 m wide by 16 m long. Treatments were 2 weed species in main plots (*A. retroflexus* and *C. album*), weed density in sub plots (2, 4 and 8 plant per meter of row) and relative time of weed emergence in sub-sub plots (8 and 4 days prior to potato and the same time with potato emergence in 2004 and the same time to potato, 2 weeks and 4 weeks after potato in 2005). For each year and location, primary tillage consisted of spring disking followed by field cultivation before planting.

Potato was planted in constant density (5.33 plants per m^2) in May 26, 2004 and May 27, 2005. In each replication 3 plots allocated to monoculture of potato, *A. retroflexus* and *C. album* and they used as control. Plots were consisted of 4 16-m rows with 75 cm spacing between rows. Potato seed tubers were planted 25cm apart in rows. There was one row space between sub-sub plots and 3 meters between replications. These spaces successfully prevented competition between plants of neighbouring sub-sub plots. Weeds were supplied by weed research department of Plant Pest and

Diseases Research Institute (PPDRI) and hand planted in 0.5cm depth. Weed emergence dates were recorded at the time of approximate 50% weed emergence. In the 3-4 leaf stage, weed seedlings thinned and field hand hoed to remove undesired weeds that had emerged. Two center rows used for data collection. Potato and Weeds were sampled at 2-week intervals. After flowering, potato plants were hand harvested in each sub-sub plot. Tuber dry weight in every 2 weeks measured and finally tuber yield in each plot calculated. Fresh weights of tubers from the 2 middle rows were recorded. For each plot in each replication, percentage yield loss was calculated as (*Y*WF - *Y*D)/*Y*WF × 100%, where *Y*D is the yield under that treatment and *Y*WF is weed free yield (weed density = 0).

Statistical Analysis

Weed density models

Hyperbolic regression model has been widely used to characterize the influence of weed density (Cousens, 1985b).:

$$Y_L = \frac{ID}{1 + \frac{ID}{A}}$$

Where Y_L is percent yield loss, D is weed density (expressed as plant/meter of row) I is percent yield loss when $D \rightarrow 0$ and, A is the upper asymptote or maximum yield loss ($D \rightarrow \infty$).
Other common way to represent weed-crop competition is the exponential model (Kim *et. al*., 2002; Scursoni & Satorre, 2005). The exponential model is represented by (Calvo *et al*., 1994):

$$Y = A * (1 - \exp(-b * D))$$

A is the percentage maximum yield loss for the first emergence time, and *b* is the rate of change.

Density-relative time models

Equation 1 has been modified to introduce the time of weed emergence (Cousens *et al*., 1987)

$$Y_L = \frac{ID}{e^{CT} + \frac{ID}{A}}$$

T is the time of weed emergence relative to the crop; *C* is the rate at which *I* decrease exponentially as *T* becomes larger. The larger the value of *C*, the more competitive the crop is against late-emerging weeds.
Other model to include the time of weed emergence has been formulated by Fu & Ashley (2006)

$$Y_L = \frac{ID}{e^{CT} + \frac{ID}{Ae^{-BT}}}$$

Equation 3 assumes that the maximum percentage yield loss (*A*) does not change when the time of weed emergence relative to the crop increases. To account for the effect of relative time of weed emergence on *A*, *A* could be specified as a function of T. It is inappropriate to make *A* linear function of *T* because a negative estimated maximum yield loss could be possible. With the same arguments characterizing the effect of relative time of weed emergence on *I* (Cousens *et al*. 1987), an alternative is to assume that *A* decreases exponentially with *T:*

$$A' = Ae^{-BT}$$

where A' is the percentage maximum yield loss at time *T, A* is the percentage maximum yield loss for the first emergence time (*T*= 0), and *B* is the rate of change of *A* with *T.* Hence Equation 3 could be rewritten as equation 4
As *D* approaches infinity, the maximum yield loss goes to *A* at *T*= 0 and goes to Ae^{-BT} at time *T* = *t* (Fu

& Ashley 2006).

Model comparison

Akaike Information Criteria (AIC) was used to compare models (Burnham & Anderson, 2002). The Akaike information criterion (AIC) is calculated for each model as:

$$AIC = -2\ (\text{log-likelihood}) + 2k$$

Where k is the number of estimated parameters in the model. A lower value indicates the "better" model. For small sample sizes, the corrected Akaike Information Criterion (AIC_c) should be used:

$$AIC_c = AIC + (2\ k\ (k+1)) / (n - k - 1)$$

Where n is the sample size. Although the best model can be selected with the lowest AIC value, it can be very useful to rank the models to know if another model is also plausible among the group of models. The models can be ranked using the Δ_i, which is calculated as;

$$\Delta_i = AIC_c - \min AIC_c$$

Δ_i can easily be interpreted with the following rule of thumb (Burnham & Anderson, 2002): models having $\Delta_i \leq 2$ are strongly plausible, values between 3 and 7 indicate that the models are less plausible, whereas model having $\Delta_i > 10$ indicates that the model is very unlikely.

Akaike weights (w_i) provide another measure of the strength of evidence for each model:

$$w_i = \exp(-\Delta_i/2) / \sum \exp(-\Delta_i/2)$$

The Akaike weights allows to know the probability that a model is the best among the whole set of candidate models.

We fitted equations to data using the least squares estimation method, assuming a normal error distribution, with the PRISM nonlinear procedure (Prism Program, Graphpad Software Inc. San Diego, USA, 2005) and Sigma Plot (SYSTAT Software Inc. 2001). Adequacy of the model fit was examined using residual analysis and R^2 criterions.

Results and Discussion

Weed density models

The models examined in this paper provided a good description of competition between the two weed species and potato yield losses as indicated R^2 (Table 1). Parameter estimations are shown in Table 1.

According to the method of AIC for model selection, the best model for *A. retroflexus* density was the hyperbolic model (equation 1) because the AIC_c value was the lowest (Table 2). Only in one case, the third emergence time of lambsquarter in 2004, the exponential model had smaller AIC_c value (Table 2). However, the exponential model was strongly plausible because $\Delta_i \leq 2$ in the rest of the cases (Table2) (Burnham & Anderson, 2002). The average w_i of 0.62 for the hyperbolic model indicates that this model has a 62 percent of probability to be the best model.. These results are according to hyperbolic equations proposed by Cousens (1985) as a general model of crop-weed competition.

C. album presented similar results that *A. retroflexus* with the hyperbolic model presenting the lower AIC_c in all the cases, except in 2004 (Table 2). The exponential model was plausible as a model to represent the relationship between C. album density and potato yield, except in 2005 (E2) where the value of Δ_i was > 10 (Table 2), indicating that in this specific case the model is unlikely. These results are confirmed for the average w_i of 0.73 for the hyperbolic model that indicates a 73 percent of probability to be the best model.

Our results shown that hyperbolic model is appropriate to represent the relationship between *A. retroflexus and C. album* densities and potato yield losses.

Parameters *I* did not vary among years for lambsquarter. Other studies also have shown that this parameter is constant among years and locations (Dieleman *et al.*, 1995; Bosnic & Cousens, 1985a). But, for redroot pigweed, this parameter decreased from 2004 to 2005, especially for E1 (table 1). In the 2005, the intervals between emergence times increased to 2 weeks (instead of 4 days intervals in 2004) and interference period between weeds and potato decreased (because of changing the emergence time treatment). In all cases parameter *I* estimated for redroot pigweed was bigger than lambsquarter.

On the basis of *I* estimation, redroot pigweed was the most competitive weed species in 2004 and 2005. Parameter *I* in redroot pigweed in 2004 was 84.19, 58.29 and 83 percent more than *I* values estimated for lambsquarter. These values in 2005 were 37.28, 20.1 and 54.8 percent. *I* parameter for redroot pigweed changed 21.5 percent among 2 years while this variation for lambsquarter was 5.1 percent

Maximum yield loss (A) for redroot pigweed was bigger than lambsquarter in 2004 and 2005. This parameter for redroot pigweed in 2004 in E1, E2 and E3 were 63.48, 60.25 and 57.65, respectively. Parameter A for lambsquarter was 57.19, 49.66 and 32.38 (table 1). Estimation of parameter A did not vary among two years for both species (table 1). For redroot pigweed this parameter estimated higher in 2005, while for lambsquarter parameter A decreased in 2005. According to the estimated parameters, maximum yield loss is related to E1 in both years. It means when weed emerge sooner than potato, they are more competitive and cause more potato yield reductions.

Tuber yield was affected by weed species, density, and emergence time. As a average, redroot pigweed caused 50.33% potato yield reduction more than lambsquarter. 2, 4 and 8 plant of redroot pigweed per meter of row reduced potato yield 15.72, 28.55 and 36.04 percent in 2004 and 13.18, 23.05 and 31.85 percent in 2005. These values for lambsquarter were 9.73, 18.84 and 25.09 percent for 2004 and 8.28, 15.17 and 21.59 percent for 2005 . VanGessel & Renner (1990) indicated that that one redroot pigweed per meter of row reduced marketable tuber yield 22-34%. When density increased to 4 plants per meter of row, potato tuber loss was 40%.

Also, emergence time affected Tuber yield, when redroot pigweed emerged 8 days before potato (in 2004) tuber yield reduced 33.76%, while in the third emergence time (the same time to potato) potato yield loss was 20 percent. These values for lambsquarter were 23.88 and 11.02 percent in 2004, respectively. In 2005, by delaying the weed emergence, yield loss decreased. Redroot pigweed caused potato yield loss 30, 22.7 and 15.4 percent in 2005 in three emergence time. But, for lambsquarter the yield loss were 21.2, 15.9 and 7.9 percent, respectively .

Weed density - relative time of emergence models

Parameter values estimated for the two models were very similar for the two species in 2004 and 2005 (table 3). In comparison between density-relative time of emergence models, in all cases, hyperbolic model (eqn 3) had smaller AIC_c values and higher weight (ω_i) in the two years and was better model to fit the data (table 2). However, the exponential model (eqn 4) was also plausible for both *A. retroflexus and C. album (t*able 2).

The difference between results of this study with Fu & Ashley (2006) is related to fit the models to the data. In this study we fit all 3 emergence times to one model , while Fu & Ashley (2006) fit each emergence time separately. They estimated parameters for each emergence time

Late emerging weed generally resulted in less potato yield loss (Cousens, 1985a; Kropff, 1988; Chikoye *et al.* 1995; Bosnic & Swanton 1997; Steckel & Sprague 2004; Fu & Ashley, 2006). From E1 (weed emergence 8 days before potato in 2004 and the same time to potato in 2005) to E3 (the same time to potato in 2004 and 4 weeks after potato in 2005) potato yield increased. Potato yield loss in response to redroot pigweed density and relative time of emergence is higher than lambsquarter. This reduction for 2004 is more than 2005. The maximum yield loss ranged from 40.11% for late emerging lambsquarter in 2005 to 63.48% for early emerging redroot pigweed in 2004. Yield reduction in second year (2005) decreased because of shorter interference period between potato and weeds (table 1&2).

Reductions in potato tuber yield with increasing in competition duration of quackgrass (*Elytrigia repens*), green foxtail and mixed population have been reported (Ivany, 1986; Nelson & Thoreson, 1981; Wall & Friesen, 1990; Love *et al*., 1995).

In other studies also, indicated that by emerging weeds sooner than potato and increasing interference period, yield loss will be higher (Nelson & Thoreson, 1981; Thakral *et al*., 1989; Wall & Friesen, 1990; Vangessel & Renner, 1990)

Time of emergence of the weed relative to the crop was also important in describing the relationship between barnyardgrass (*Echinochloa crus-galli* L.) and corn (*Zea mays* L.) yield (Bosnic and Swanton 1997), redroot pigweed (*Amaranthus retroflexus* L.) and soybean [*Glycine max* (L.) Merr] yield (Dieleman *et al*., 1995), and wild oat and wheat or barley yield (O'Donovan *et al*., 1985)
In this study hyperbolic model had better results for fitting density and density-relative emergence time data. However, Exponential model can be presented as a suitable model to represent the competition with potato.

Our study determined that time of emergence time and density of redroot pigweed and lambs quarter affected potato yield. Early emerging redroot pigweed and lambsquarter can be highly competitive with potato.
Results of this study can be incorporated in integrated weed management program for potato. It could serve as the basis for making economic decision rules for managing redroot pigweed and lambsquarter in potato crop and develop weed thresholds for Iranian farmers.

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THE INFLUENCE OF VARIETY, PLANT DENSITY AND DOSES OF FERTILIZER ON YIELD AND QUALITY OF TUBERS

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INTRODUCTION

In Republic of Moldova potato as a food crop play an important roll. During the last few years a positive change took place. A number of new, high, productive varieties, where listed, more and more farmer use for planting high quality seeds and modern equipment for potato production. Productivity and quality of yield increased, and made potato an attractive business culture. There are many professional farmers which obtain 30-40 t/ha, and successful grow potato for early market or winter consummation. Taking into the consideration producers intention to increase yield per ha and consumers requirements for better quality potato, future developments in potato production need a supplementary knowledge's.

It is well know that variety, plant density and fertilizer can sufficiently influence the yield and quality of the tubers.

The importance of variety and influence of fertilizes on yield and quality of potato has been studied separately in the recent past (Iliev P. & Iliev I., 1999, 2005 A.Veerman, P. Struik, C. van Loon, 2002; Iliev P., 2005), but no studies about plant density in combination with mentioned above factors.

The objective of the experiments was to examine, how tuber yield and quality of different varieties could be optimized by different amounts of mineral fertilizer and plant density.

MATERIAL AND METHODS

The experiment started in 2004 on black soil (pH 7,0 - 7,2), with the content of humus in the profile (0 - 30 cm) – 2,2 - 2,5%, available nitrogen 40 - 55, mobile phosphorus 65 - 80 and exchangeable potassium 314 - 334 mg/kg of dry soil, as a three factorial experiments to examine the effect of plant density (45000, 50000 and 55000 plants/ha), doses of mineral fertilizer (NPK – 0, NPK – 60, NPK – 120, NPK – 180) on variety reaction of three group of maturity (early, second early – medium, and medium – middle late varieties).

Each variant was planted in 4 replication. Fertilizers were applied by broadcast before preplanning soil preparation. During the vegetation period we applied, in dependence of year conditions, 3 - 4 irrigation by sprinkle irrigation keeping the soil humidity at the 75 - 80% relative soil humidity Time of planting: middle – end of March. Soil samples have been taken to a depth of 30 cm from each replication, before fertilizer application. Starting 10 days after emergence plant and soil samples have been take 4 times at the main vegetation stages – beginning of tuber ignition, period of intensive tubers grow and before harvest. Plants of each plot were dug for examination: on haulm – number of main stems and underground branch, leaf area, fresh and dry weight of haulm, length of stems. In tubers: the total number, size and dry matter and starch content, ascorbic acid, sugar, and nitrate content. After harvest boiled tuber were tested to flash darkens.

REZULTS AND DISCSSION

Mineral fertilization significantly increased tuber yield, but there efficiency depends of biological particularities of varieties, length of vegetation period and plant density.
Usually farmers use a 50000 plants/ha, considered as common practice. Our results show that plant density can varied in dependents of variety doses of fertilizer and destination of crop: seed production or table potato.

As show in table 1 the best plant density for early variety Agata, Flavia, Velox is 55000 plants/ha, as for Impala with more developed haulm the optimal plant density is 50000 plants/ha.

Table 1Productivity of early variety in dependence of plant density and doses of fertilizer, t/ha

Variety	Plant density, th/ha	Fertilizer doses, kg			
		NPK, 0	NPK, 60	NPK, 120	NPK, 180
Agata	45	22	31	39	41
	50	25	34	42	43
	55	27	38	45	46
Flavia	45	20	29	34	37
	50	23	31	36	39
	55	25	34	39	41
Impala	45	24	33	43	48
	50	26	37	45	52
	55	27	39	47	53
Velox	45	21	27	35	38
	50	23	30	39	43
	55	24	32	41	44

Increasing the doses of fertilizer leads to yield increasing, but starting with level $N_{120}P_{120}K_{120}$ efficiency of fertilizer grow very slowly and practically has now economical efficiency on extra early varieties Agata, and Flavia . Variety Impala and Velox show a positive results, but higher doses increased yield not straightforward , efficiency is described by curve with die away effect

Second early varieties having a longer vegetation period react more efficiently on highest doses of fertilizer, but in the same time we observed an inefficiencies, when plant density increased till 55000 plants/ha. Dates from table 2 shout that the best results are obtained in the plots with plant density 50000 plants/ha and fertilizer doses between $N_{120}P_{120}K_{120}$, and $N_{180}P_{180}K_{180.}$

Tuber yield was positively correlated with leaf area and duration. The maximum yield has been rich when leaf area was 4,5 -5,0 time higher than plant nutrition and should be around 45000 – 50000 m^2/ha.

In case of insufficient leaf area tuberization process could be affected by soil hitting. For instance soil temperature in the bush on not fertilizer plot with less plant density is 3 – 4^0C higher than in well fertilized and optimal density plot.

Results clearly indicated that abundant amount of NPK delays tuber initiation and bulking rate at earlier stages in special on plots with maximum plant density, but in the some time increased the tuber number and weight per plant in the plot with low plant density and decreased the tuber size on higher density. So for earlier big size tuber yield is recommended a density of plant between 45000 – 50000 plants/ha and fertilizer application about $N_{120}P_{120}K_{120}$ or $N_{180}P_{180}K_{180}$ in dependence of variety type. For seed production 50000 -55000 plants/ha density and fertilizer doses N120P120K120 is recommended.

Table 2 Productivity of second early variety in dependence of density and doses of fertilizer, t/ha

Variety	Plant density, th/ha	Fertilizer doses, kg			
		NPK, 0	NPK, 60	NPK, 120	NPK, 180
Amorosa	45	25	31	39	45
	50	27	34	47	52
	55	28	37	45	49
Arnova	45	24	30	35	42
	50	26	33	44	53
	55	27	35	46	50
Romano	45	22	30	36	41
	50	25	34	42	43
	55	26	35	43	44
Ilona	45	20	27	34	40
	50	22	31	39	43
	55	24	33	40	41

In the group of the main crop varieties (table 3) were established that variety Desiree and Sante had the best productivity, when plant density is 45000 plants/ha and fertilizer doses $N_{120}P_{120}K_{120}$, the following increasing of plant density and fertilizer doses has now economical coverage. As for variety Kondor and Kuroda the optimal plant density is between 45000 – 50000 plants/ha applying $N_{120}P_{120}K_{120}$. In case of les density and highest fertilizer doses Kondor gives an extra large tubers from 600 till 1000 gr.

Table 3 Productivity of main crop variety in dependence of density and doses of fertilizer, t/ha

Variety	Plant density, th/ha	Fertilizer doses, kg			
		NPK, 0	NPK, 60	NPK, 120	NPK, 180
Desiree	45	23	31	39	43
	50	25	33	42	44
	55	26	34	43	40
Kondor	45	28	36	48	56
	50	31	39	55	60
	55	33	40	54	57
Kuroda	45	24	33	44	45
	50	26	36	47	52
	55	28	39	49	50
Sante	45	25	34	43	46
	50	27	35	44	47
	55	29	37	46	49

The highest fertilizer doses show insufficiently yield increasing. However on pour soils (in separated) experiments fertilizer doses should be increased till $N_{180}P_{180}K_{180}$.

Tuber dry matter yield was significantly increased by fertilizer on early variety plots, but reduce the content in tubers and haulm. On late variety plots dry mater yield decreased starting with N120P120K120 doses on all density plots. The same situations were observed on starch content.

Ascorbic acid and nitrate content on the tubers were predominantly influenced by variety and fertilizer doses. Nitrate content was significantly increased by fertilizer starting with amount of N120P120K120

In the results of our studies we set up that yield, tuber size and quality depends of variety maturity group, plant density and fertilizer doses. Moreover it could be difference between varieties in the same group of maturity dew to biological particularity of variety

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REDUCING COSTS CAUSED BY ISOLATION REQUIREMENTS BETWEEN GM AND NON-GM POTATO FIELDS – A METHOD BASED ON GIS

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Requirements for cultivating GM potatoes include isolation from non-GM potatoes. The Finnish Ministry of Agriculture and Forestry released its final report on the enabling of co-existence between genetically modified crops (GM) and conventional (non-GM) crops at December 2005. Requirements for co-existence for potato are: 1) Seed for internal supplemental seed use must be grown on fields where GM potato has not been produced, if GM-free potato is to be produced; 2) the equipment must be cleaned very carefully if they are used by several parties and in areas where genetically modified potato is cultivated; 3) Isolation distances 5–10 m[1]; 4) residual vegetation must be collected and destroyed; 5) after GM potato, non-GM potato may be cultivated on the field only after a year of non-potato cultivation, and certified seed potato must then be used as the seed and 6) retail finishing and sorted tubers must be handled appropriate so as to prevent the mixing of varieties
Isolation requirements of GM and non-GM potato cause costs. The costs are caused, for example, by border strip requirements, that reduce the production area of the cultivated plant on the field, and another plant must be cultivated between two potato fields to reduce the risk of pollen and tuber spreading. Costs are also caused by the cultivation rotation requirement: farmers must cultivate a plant other than potato during the transition years from GM varieties to non-GM varieties. To evaluate the costs caused by the border strip requirement, we need information on how the fields used for potato cultivation are located with respect to each other, what their regional concentrations are, what their shapes are and whether there are natural or man-made border strips (roads, bodies of water or forests) between the fields. To evaluate the costs incurred by the cultivation rotation requirements, we need information on how the farmers presently carry out their cultivation rotation. Some of the farms only cultivate potato, while others may have sufficient field area to implement cultivation rotation but potato production is still concentrated. This may be owing to, for example, the fact that not all fields are suitable for potato cultivation. Several studies indicate that intensive production is focused around the farmhouse of the farm (e.g., Myyrä 2001). The potato transportation volumes are high and therefore potato production may have been concentrated near the farmhouse. The cultivation rotation requirements may increase the transportation distances to the potato fields, which increases production costs.

Data and methods

The research data in this study is comprised of four different materials: Map data from the GIS location information system, the field-map information register of the Ministry of Agriculture and Forestry's information management centre (TIKE), the Finnish Food Safety Authority (Evira) protocols and the profitability monitoring of farms with profitability accounting. The information from farms with profitability accounting has been supplemented with farm model calculations.

By means of the inspection material of Evira we can carry out the correlation between distance of two potato field and the presence of foreign varieties in fields with a distance of 20 metres or less. A non-parametrical Spearman correlation coefficient has been calculated for this correlation. The correlation coefficient is very small (-0.03) and does not have statistical significance (p=0.15). This indicates that no statistically significant connection between the distance and presence of foreign varieties can be detected. Neither has the cultivation history of previous years nor the size of fields had statistically significant impact on whether foreign varieties could be found on potato fields.

In this study, we assess how the shape, size and distance to other potato fields affect the need for border strips around potato fields, and what are the effects of this on production costs. As factors

[1] Regulations will be verified after ESGEMO-research program.

affecting costs we consider the proportion, number and type of GM varieties in certain area, climate, shape of fields, crop rotation, structure of farmland ownership, and landscape structure. We assess this by means of a GIS analysis. Each farm has a different field configuration, determined by the neighbouring fields and surrounding landscape. The pattern of ownership may be such that co-operation between farmers is needed to provide a feasible solution. With GIS we can take these different factors into account, and optimize the location of GM and non-GM potato fields so that the costs of co-existence are minimized.

Based on GIS, fields are inspected square by square as quarters of the basic map (5 km x 5 km). Figure 1 presents an example for one basic map quarter. The figure presents the location of the potato fields of a farm in Southern Ostrobothnia, the most dense potato cultivation area in Finland. This region is characterised by long and narrow fields. The figure outlines the fields administered by the farm in question. Dark grey colour is used for fields where potato is being cultivated during the growth season in question, while lighter grey is used for neighbouring farms' fields that are or may be used for cultivating potato. The farmer has insufficient information of what the neighbours are cultivating. White colour indicates fields with no potato cultivation during the last five years in review. A grey dot is the farmhouse of the farm in question.

Figure 1. Location of potato fields

Figure 2 shows four farms. Outlined fields are used by the farms for potato cultivation during a specific growth season. The border strips of the fields are indicated in dark grey in the figure. Border strips are required when GM varieties are cultivated in fields. In certain fields of circular or rectangular shape and with natural border strips, even smaller border strip requirements may suffice, which long and narrow strips may render potato cultivation almost impossible.

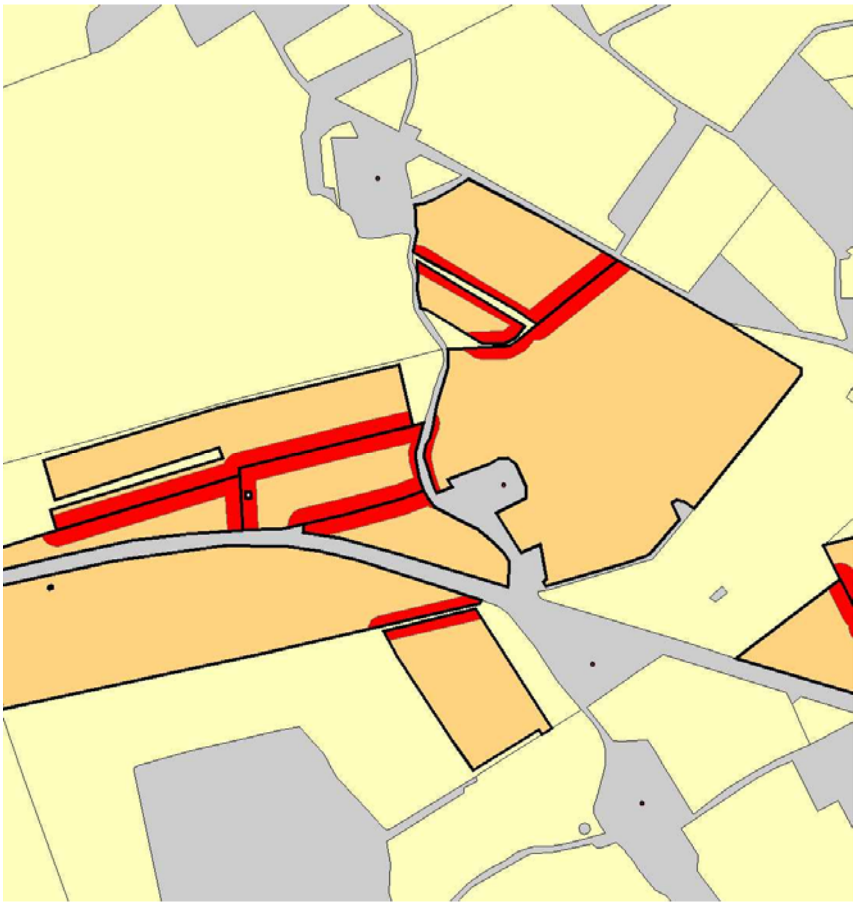

Figure 2. Border strip requirements

Results

In food potato production, the problem is monoculture and the concentration of cultivation on a small area, although it should be noted that seed potato production is even more concentrated from the area perspective. The production cost of food potato is ¢25.93/kg (ranging from ¢25.21 to ¢30.31 per kg) in monoculture when the additional seed is produced on the same farm. If food potato were produced with the same production requirements as seed potato to avoid variety mixing, it would increase the production cost of food potato by ¢7.71/kg (30%) for each potato farming year. Transportation cost of imported potato from Sweden to Finland wary between ¢1.5 – 3/kg.

Cultivation rotation requirements

If the isolation requirements call for cultivation rotation, the farm's business becomes non-profitable. The requirement of even one year of keep from cultivating potato when moving from GM potato to regular (non-GM) potato farming renders farming unprofitable during the transition year in an average-size (37.50 hectares) full-time farm. One year of keep from cultivating potato, during which time cereal is cultivated on the parcel, increases the production cost of food potato by ¢8.58 (24.9%) (¢4.53–¢11.37 per kg) in monoculture if the supplemental potato seed is cultivated on the farm. In other words, if a farmer cultivates GM potato and then changes to non-GM potato after one year of pause, during which time the farmer has cultivated cereal on the parcel, the production cost of food potato is ¢8.58 higher during the first potato cultivating year than it would be without the one-year pause. Two years of keep from cultivating potato increase the cost of production by ¢16.72/kg (39.2%) (¢8.77–¢22.46 per kg).

Border strip requirement

The need for border strips varies regionally. In areas with lower proportion of potato production, with regular-shaped fields and natural border strips around the potato fields, the need for in-field border strips is lesser. In the Southern Ostrobothnia region where potato production is more concentrated, the need for border strips is greater, which generates more costs for the farmers. For example, a 10-metre border strip in Northern Ostrobothnia would cause an average ¢15 per kg (0.6%) (¢0.10–¢0.80 per kg) additional cost per potato-kg while the additional cost in the Southern Ostrobothnia region would be ¢0.37 (1.4%) (¢0.25–¢1.84 per kg). The essential, however, is that potato producers may not know what plans the neighbouring farm has for the adjacent field. The deployment of genetic technology may increase plant alteration in the neighbouring farms in that they, too, may commence potato cultivation in fields where they have not previously done so. In this case, the uncertainty regarding the border strip requirement and the costs thus incurred may rise to considerable amounts. In this case the premise of calculation is that, there is a border strip at each end of the field. Then a 10-metre border strip increases the cost of production by ¢2.34/kg (9.0%) (¢1.41–¢3.60 per kg).

<u>Economic impact of potato field distances</u>

Because of cultivation rotation and border strip requirements, farmers will need to give up potato cultivation in fields where they have carried out potato-monoculture. As potato production is often concentrated near the farmhouses, the deployment of new fields contributes to transportation costs. If potato were to be cultivated at the same distance as other plants in average, it would increase the cost of potato production by ¢0.15 per kg (0.6%) (¢0.1–¢0.48/kg).

Conclusion

There is no cultivation rotation requirement if the farmer always continues to cultivate GM potato in the same field that had GM potato in the preceding year. However, if the farmer wishes to cultivate non-GM potato on the field after GM potato, a potato-free year must occur on between the potato types. Even one-year cultivation rotation requirement after GM potato is expensive to implement for farmers. It would lead to a situation where it would no longer be economically profitable for farmers to revert to cultivating non-GM potato in a parcel after GM potato has been cultivated. This would result in a competitive advantage for continuing GM potato cultivation. On the other hand, awareness of sunk costs and uncertainty (or risk) of the costs incurred may prevent farmers from adopting GM potato in the first place.

Increasing the cultivation rotation forces the farmers to cultivate potato on fields further away from the farmhouses, which increases transportation costs. Potato production is characterised, by transportations of greater harvests than in crop cultivation and by several pesticide sprayings during the growth season. This is one of the reasons contributing to the concentration of potato cultivation in the proximity of the farmhouses.

A border strip of 10 metres is expensive to implement for farmers. If a border strip requirement is imposed on a farmer that cultivates GM potato, the costs are attributed to the GM-potato-producing party. This would cause a competitive advantage to farmers that cultivate non-GM potatoes. It would be worth considering whether the same result could be attained with a smaller border strip, considering particularly Eviras inspection material, which indicates that no statistically significant connection between the distance and presence of foreign varieties can be detected. Neither has the cultivation history of previous years nor the size of fields had statistically significant impact on whether foreign varieties could be found on potato fields.

The farmer can always state that the products produced may contain genetically modified material and in that way avoid seed test requirements. However, in such cases, the farmer will always find it profitable to cultivate GM varieties as they have lower production costs. If the farmer wishes to produce non-GM potato and market it as a non-GM product, the segregation costs may rise too high.

The essential is that potato producers may not know what plans the neighbouring farm has for the adjacent field. A farmer has imperfect information. The deployment of genetic technology may increase plant alteration in the neighbouring farms in that they, too, may commence potato cultivation in fields where they have not previously done so. In this case, the uncertainty regarding the border strip requirement and the costs thus incurred may rise to considerable amounts.

The deployment of gene technology contributes to the networking of the supply chain: The GM seed will arrive on the market through variety representatives. The plant breeder of potato varieties will collect part of the benefits through chains. Even today, the Finnish potato supply chain appears quite integrated: 67% of the Finnish seed potato market is covered by the two largest strain representatives. (Tuomisto 2007). The stricter the variety mixing limits for non-GM potato, the more the variety representative (Principal) can benefit: It is more difficult for farmers (Agent) to discontinue GM potato production if the variety mixing limits are high. Variety representatives or cultivation licensors that may be large actors on the market have better capacity than individual farmers to use a GIS system to assist in locating fields while they can lower the costs caused by parallel existence. The cultivation licensor may collect some of the benefits to itself using this method. On the other hand GM potato must also benefit the producer. Otherwise GM varieties will not be introduced on farms. (Tuomisto 2004)

The most problematic issues are handling of waste potato, potato cultivation on domestic parcels for household use and the implementation of internal seed regeneration on the farm. Moral hazard problems may arise in connection with the implementation of measures needed for the coexistence of GM and non-GM potato. A food potato producer may sell GM potato directly from the farm for food potato use or as seed for domestic-use parcels as non-GM seeds. (Tuomisto 2007; Tuomisto 2004; Salanié 1997).

The stricter the farming requirements are set, the higher the costs will raise and, consequently, the more imported potatoes or imported potato products (include GM products) gain competitive advantage; transportation costs of imported potato products would be lower than the cost of co-existence of domestic potato production.

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Precision and Organing Farming

INTEGRAL AND SPATIAL-DIFFERENTIATED AGROTECHNOLOGIES OF EARLY POTATO IN PRECISION FARMING

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Potato is essential food product and necessary for people of the Republic of Bashkortostan all the year round. Even stored under the best conditions, potato loses some quality the longer they are stored. A lot of essential nutrients are lost in the breathing process. Fresh potatoes with high nutrition value should be used for full value ration. Climatic condition of the Republic of Bashkortostan are not suitable for growing potatoes all the year round, but it is quite possible to get adequate amount of fresh potato from June. Nowadays potato growing in the Republic of Bashkortostan has been focused in private and small market farms (97% of all planting area). It is grown on the area of 3,000 hectare in large market farms (table 1) and on the area of 1000 hectare on peasants farms. Potato consumption in the Republic of Bashkortostan has increased to 180 kg per capita for recent 3 years.

The authors of the article as the basis of the integrated agrotechniques of early potato used knowledge of natural stable produce process in agrophytocenose for optimizing all its constituents and increasing planting productivity and tuber yield quality. Productivity of early potato is understood as a possibility of early potato to produce maximum output of dry matter per area unit by the concrete date of summer harvest. The constituents of dry matter are starch, protein, vitamins, mineral salts. It is necessary to study the productivity of early potato in connection with conditions of germination and its individual components. The chief criterion of early potato productivity is tuber yield capacity.

We found out that during the period of intensive accumulation of early potato yield the amount of tubers increased to the end of July and then it didn't change. But the amount of the commercial tubers gradually was increasing to finish of the harvesting. Thus, yield accumulation in July and early August occurs due to increasing amount and mass of commercial tubers. Two periods can be singled out in the tuber formation process. The first one is the formation of vine, growth of stolons and beginning of tuber formation. The second one is increasing tuber mass due to functioning of overground mass and root system. Average mass of tubers will be larger. Besides it was found out that growing of high plastic variety ensures yields of high quality. Different early ripening and medium-early varieties differ response to growing conditions in different periods of vegetation.

Increasing early potato production in the Republic of Bashkortostan will occur by means of crop capacity increase and tuber quality. Applied up to present day's chemical-technogenic and bioorganic alternative agrotechniques cannot meet the needs of the population with fresh potato. New agrotechnique development represents a constant process and its pattern is not be used. The bases of new proper agrotechnique formation of early potato are farming rules, biochemistry and potato crop physiology, establishing and practicing of long-term multifactors field experiments.

That's why for every farm certain level of early potato crop capacity must be defined to provide high economical and power production effectiveness. The whole range of peculiarities should be taken into consideration in crop production. 1. Biological peculiarities. To receive an early product the varieties of early tuber formation and active tuber accumulation are most valuable. 2. Soil and climatic conditions of farm area landscapes. In the Republic of Bashkortostan black soils and grey forest soils of heavy and medium loam mechanic composition prevail. 3. Variety differences. In depend of requirements of consumers it is necessary to grow the varieties with different market properties. 4. Mastering of techniques, accelerating plant growth and development and tuber yield accumulation. The main criterion of applying agrotechnology properness is their complexity and differentiation due to soil – climatic and organization – economic conditions, biological peculiarities of early potato culture while conserving soil fertility and ecological safety and obtaining planned yield in calculate date.

Integrated agrothechnology formation scheme of early potato crop in the Republic of Bashkortostan is founded on equality and indispensability of the following crop production factors: 1. Ecological conditions and plant growing location. 2. Organizational and economical conditions of producers. 3. Integrated agrotechnique recommendations. 4. Specialized crop rotations. 5. Organic-mineral nutrition and differential plant irrigation. 6. Biologizised plant conservation system from diseases and insects. 7. Reclamational different depth layer soil cultivation. 8. Specialized farm machinery. 9. Selection and seed-growing of adapted home varieties of early and medium group's maturity. 10. Qualified agrobioenergrtic estimation of agrotechnique effectiveness.

Agrotechnology improvement should be directed to making proper plant growing conditions, yield increasing and quality improvement of early potato fresh tubers through management of production processes. This aim can be achieved by formation of integral and spatial-differentiated agrotechnologies in the system of integrated and exact farming. We can give the following definitions to integrated agrotechnologies and integral agrotechnologies of potato growing. Integrated agrotechnologies is the state of connecting of all parts, sections and functions in potato growing agrocomplex system. This state of connection provides a high level of their differentiation taking into account the use of finished products, biological features of varieties and also leads to their consolidation as the aggregate to form optimal agroecological and agroproductive characteristics of farm area landscape and planned tuber yield production. Integral agrotechnologies are the direct step to spatial-differentiated agrotechnologies of early potato in the Republic of Bashkortosan, because they require irreproachable maintenance of technological disciplines. All agrotechnical methods are interacted and interconnected. Any parameter changes in the first operations influence parameter changes in the following operations. Integral agrotechnologies of early potato are complex dynamic system of agrotechnical methods. These methods are differentiated to specific soil-climatic, organization-economic conditions of market, state and private farms in the Republic of Bashkortosan. Also these methods are integrated into optimal complex, where every technological operation can help to bring out potentialities of early potato and production of planned tuber yields. We can exactly determine the quantity of planned yields depending on resources provided. The amount of mineral, organic and organic-mineral fertilizers is determined on the basis of soil and plant analysis. On the basis of monitoring we take a decision to regulate the application of protection measures and optimization strategy of phytosanitarian state of early potato agrophytocoenosis.

There are different ways to decrease energy consumption in integral agrotechnologies of early potato. 1. Planting stock of high quality. 2. Passing to local application of mineral and organic-mineral fertilizers with balanced macronutrients and micronutrients. 3. Mastering the use of continuous crop rotation using catch crops and biological reclamating substances for partial and full change of organic fertilizers. 4. For plant protection from pests we can use not only chemical preparations but also the complex of biological means, agrotechnical and other operations depending on the degree of disease, pest and weed development.

In forming agrotechnologies of exact farming in farm area landscapes we examine every agrophytocoenosis with its spatial non-uniformities of meso-and micro-relief, dynamics of edaphic conditions along the slope and soil horizon. A large data base, constant monitoring of field condition, modern programme ensuring allows to apply differentiated management to production cycle of crop cultivating and harvesting. Owing to it we can achieve as high resource and energy saving as possible, increase of labour productivity, decrease of farm product cost-price, and the chief thing is to optimize all production process factors. The system of automatically taking decisions is the first and the last link of exact farming management (SATD). Thanks to this system we make technological schedule for carrying out all technological operations. Parameters of necessary technological operations are inserted into the memory of electronic robot-technical device.

In order to work out spatial-differentiated agrotechnologies of early potato it is necessary to carry out the following preparatory operations. 1. Make a selection according to proper methods of soil tests with spatial connection to local co-ordinates. 2. Determine all necessary agrophysical and agrochemical soil indices. 3. Determine littering, disease, pest and useful ento-fauna spreading and other indices of farm area landscape state. 4. Make schedules and maps on the basis of these indices. 5. Work up, systematize, analyze and draw conclusions that it is necessary to apply the complex of agrotechnical and organization- controlling methods. 6. Devide rotation fields into different size plots. These plots must have similar indices with their exact co-ordinates within the bounds of dynamics intervals.

The stages of working out and introducing spatial-differentiated agrothechnologies of early potato may be listed in the following order. 1. Choosing the productivity level of early potato. By our data we can get 110 t/ha of early potato tubers in the Republic of Bashkortostan. During our experiments we used integral agrotechnologies in bodharic conditions and productivity reached to 40-70 t/ha, when we used irrigation our productivity reached to 70-90 t/ha. 2. Making data base of crop rotation and agrophytocoenosis on electronic and paper medium on the basis of scientific research and production data. 3. Forming electronic maps of farm area landscapes. 4. Analyzing obtained information about the level of early potato agrotechnology intensity in this organization economic and soil-climatic condition. 9. Making spatial-differentiated agrothechnologies of early potato for concrete rotation field. These agrothechnologies are applied after the proper predecessor, using the best crop variety for this farm and necessary farm machinery. 6. Professional training and constant studies of all workers employed in producing, storing and marketing of early potato. 7. Forming and inserting necessary technological operation parameters into the memory of electronic robot-technical devices. 8. Control at carrying out of current technological operation and correcting parameters of the following technological operation according to the change of early potato agrolandscape state. This control is carried out by means of video-shooting using different types of flying machines and space satellites.

Multifactor field experiments on investigating different integral agrothechnologies of potato were carried out on leached black soil in irrigation vegetable rotation. These experiments took place on the state farm “Alexeevsky” Ufimsky district, the Republic of Bashkortostan in 1995-1998 and in 2001-2005. Planting stock was let germinate during25-30 day depending on experiment variants. Potato was planted to a depth of 6 to 8 cm, at soil temperature in tuber planting depth + 6-8ºC in early May. Under autumn ridging we planted potato with forestalling in 4-5 days, under spring ridging we planted potato with forestalling in 1-2 days. Planting thickness of the highest-quality potato depending on spring moisture supply is 50-55 thousand tubers per 1 hectare. Planting scheme: 70cm·28,5 and 75·26,7.

Field experiment plan included the following variants. A Factor. Planting method and soil cultivating system, soil surface grading and planting care. 1-2. Level-field system of planting with row width 70cm. 1. 1.1. Preplanting soil cultivation. Early spring harrowing of late-fall plowed field to 4-5cm БЗТС-1, mellowing to 22-24cm. Rototilling of soil to 14-16cm, level-field system of planting. 1.2. Care. Before the potato plants have emerged harrow the soil 2 times and after seedling emergence harrow once more, then cultivate to 12-14cm and 10-12cm and hill up. As in variant 1 + use shallow cultivation to the depth of 4-5cm after each watering before vine closing. 3-4. Semiridge planting with row width 70cm. 3. 3.1. Preplanting soil cultivation. Early harrowing of late-fall plowed field to 4-5cm БЗТС-1, mellowing to 22-24cm, rototilling to 10-12cm, semiridge planting with ridge height to 10-12cm. 3.2. Care. Before the potato plant have emerged cultivate the soil two times by three-tiered arrow-shaped teeth with rotation scarifiers designed by Gurianov P.V. (experimental training farm “Milovskoe” FSET HPE BSAU). After seedling emergency cultivate once more by three-tiered harrow-shaped teeth with rotation scarifiers and cultivate once more without rotation scarifiers and then hill up. As in variant 3 + use shallow cultivation to the depth of 4-5cm after each watering before vine closing. 5-6. Ridge planting with row width to 75cm. 5. 5.1 Preplanting soil cultivation. Early spring harrowing of late-fall plowed field to 4-5cm БЗТС-1, mellowing to 22-24cm, profile formation of volume ridge ZIRKON 7/300 and ridge planting VL 20 KLZ. 5.2. Care. As in variant 3 use shallow cultivation to the depth of 4-5cm after each watering before vine closing. 7-8. Ridge planting with row width to 75cm under autumn ridge cutting without spring smoothing. 7. Preplanting soil cultivation. Autumn cultivation to the depth of 12-14cm takes place in September. It is done by heavy cultivators like КПЭ and КПШ. Deep mellowing to the depth of 30-32cm and volume ridge formation ZIRKON 7/300 take place at the end of September. Ridge planting will take place next spring. As in variant 5 + use shallow cultivation to the depth of 4-5cm after each watering before vine closing. B Factor. Fertilizer system. Control is necessary if fertilizers are not used. With fertilizer doze 30 t/ha it is necessary to apply nitrodiammophos and potassium sulphate which are common mineral fertilizers. With fertilizer doze 40 t/ha of rotted manure, mineral fertilizers, modified micronutrients in chelating form Kemira Universal-2 which are organic-mineral fertilizers. Rotted manure is applied in spring only in the case of autumn ridge cutting. Under level-field system of planting and semiridge planting we apply fertilizers by broadcasting method AMAZONE ZA-M MAX using deep mellowing. Under ridge planting mineral fertilizers are applied by local method. When we don't irrigate the soil we apply the half of mineral fertilizers for broadcasting method by local method. C Factor. Irrigating control is necessary if you don't irrigate. Sprinkling irrigation with prewatering threshold in crop development period 80-85-80% HB. I. Planting- budding beginning; II. Budding – blooming + 10 days; III. Blooming + 10 days – Blooming + 20 days. Rated soil layer during the first period is 50cm, then 60cm.

Table 1. Tuber yield of early potato with different Integral agrotechnologies.

State Unitary Agricultural Enterprise state farm "Alexeevsky", the Republic of Bashkortostan.

Fertilizer system	Irrigation	Seeding method and soil cultivation system			
		Level-field system of planting	Semi-ridged system	Ridged system when cutting ridges In spring	In autumn
"Nevsky" variety, 1995 – 1998.					
Without fertilizers	Without watering	21,6	21,4	21,3	21,5
	Sprinkling irrigation	26,7	26,8	26,9	26,8
Rated portion for 30tones per hectare	Without watering	25,8	25,5	25,4	25,7
	Sprinkling irrigation	34,8	34,9	35,0	34,8
Rated portion for 40tones per hectare	Without watering	28,8	28,5	28,5	28,7
	Sprinkling irrigation	41,9	42,1	42,3	42,0
LSD_{05}		2,5			
"Red Scarlet" variety, 2001 – 2005.					
Without fertilizers	Without watering	24,7	24,68	24,9	25,1
	Sprinkling irrigation	39,6	39,78	40,7	40,6
Rated portion for 30tones per hectare	Without watering	30,9	30,7	31,0	31,4
	Sprinkling irrigation	60,2	60,5	61,6	61,6
Rated portion for 40tones per hectare	Without watering	33,0	32,7	33,0	33,5
	Sprinkling irrigation	71,0	71,4	72,7	72,4
LSD_{05}		2,7			

Optimal growth conditions allow early potato plants to form rather large area of leaf surface in all variants (50…80 thousand m^2). High safety of potato plants to harvesting time and high efficiency leaf apparatus and root system absorbing surface, provided forming planned tuber yield (Table 1). Use coefficient of fertilizer nutrients during irrigating reached 90%.

According to Table 1 there is no essential difference between different planting methods and soil cultivation systems, soil surface profiling and planting care. We reached it because we provide optimal conditions for every early potato plant and agrophytocoenosis at each growth and development stage. High quality of fresh tuber yield was achieved in all variants. It is necessary to single out low content of dry matter, starch and vitamin C in Red Scarlet variety yield.

THE IMPACT OF AGRONOMIC MEASURES ON YIELD AND QUALITY OF ORGANIC POTATOES (*SOLANUM TUBEROSUM* L.) FOR INDUSTRIAL PROCESSING

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Abstract

Three field experiments were conducted during 2002 and 2004 on two sites in Germany in order to examine the impact of preceding crop, pre-sprouting, N- and K-fertilization and cultivar on total tuber fresh yields, tuber DM, glucose and fructose concentration, as well as the colour of crisps and the quality score of French fries at harvest and after storage. Generally, total tuber yields depended very much on the growing season. Highest yields were obtained when an organic N source was applied along with potassium sulphate or when the crop was cultivated after peas and grass-clover, respectively, instead of cereals. Increasing yields after cattle manure fertilization (one of three years) could be attributed to K rather than N. Combined N and K fertilization may cause DM concentration to fall short of the required minimum for crisps. Pre-sprouting and storage increased tuber DM concentration considerably. Cultivars belonging to the very early and early maturity type showed the largest relative increase of reducing sugars due to storage.

On the whole, results suggest that the effect of agronomic measures such as fertilization, preceding crop and seed-tuber preparation on internal tuber quality and quality of fried products may be rather small. In contrast, the quality standards for tuber raw stock can be accomplished best when adequate cultivars suitable for storage are chosen.

Introduction

Organic cultivation of potatoes for industrial processing into French fries or crisps may be a new source of income for organic farmers in European countries. For potato processing, high proportions of larger tubers are required for French fries and also for crisps. In addition, there are ranges and thresholds for tuber dry matter (DM), as well as for the concentration of reducing sugars (glucose and fructose) of tubers. Tubers should not only meet these standards shortly after harvest, but also after storage. Tuber size is mainly determined by nitrogen (N), and tuber yield response is mainly dependent on the rate at which N is released from preceding crops or organic amendments such as animal manures. Little is known about the potential interactions between N supply and crop growth as a function of seed-tuber preparation and cultivar. In organic farming, where N is usually very limited, the correlation between available potassium (K) - applied as mineral K or with cattle manure - and potato crop response may be low.

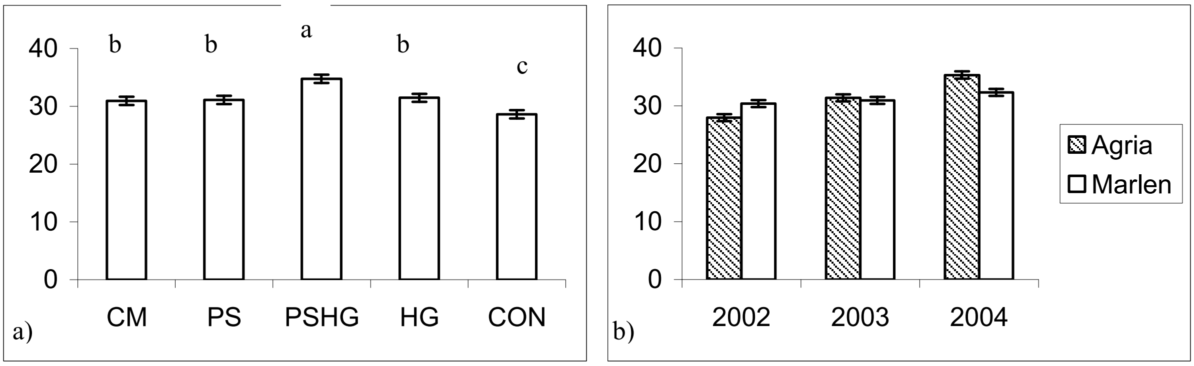
Materials and methods

Three field experiments were conducted over two and three years, respectively on two organically managed sites (DFH: 51°4, 9°4', BEL: 52°2', 8°08') in Germany in split-plot designs (Exps 1 and 2) and in a RCBD (Exp. 3) on loamy sand (Exp. 1) and silt loam (Exps 2 and 3) with factors fertilization (cattle manure, potassium sulphate, horn grits, both combined, and a control) and cultivar (Agria and Marlen) in Exp. 1, preceding crop (oats, peas, grass-clover, winter wheat), pre-sprouting (yes or no), cultivar (Agria and Marlen) and date of harvest date (two early and one final) in Exp. 2, and cultivar (see Results) in Exp. 3. Details on site and weather conditions, agronomical measures and statistical analysis are presented in Haase et al. (2007 a-c). Parameters discussed in this paper are total tuber fresh matter (FM) yields, tuber DM, glucose and fructose concentration, as well as the colour of crisps and the quality score of French fries.

Results

In Exp. 1, tuber fresh matter (FM) yield was consistently and significantly increased ($p<0.0001$) by any fertilizer treatment as compared with the control. Combined potassium sulphate and horn grits application (PSHG) gave the strongest yield response (+6.1 t ha^{-1}), while cattle manure (CM) and PS or HG alone did not differ significantly from each other (Fig. 1a). While in 2002, cv. Marlen yielded significantly higher than cv. Agria, the opposite was true in 2004. In 2003, total FM tuber yield (mean of both cultivars) was 31.3 t ha-1 (Fig. 1b).

Figure 1: Fresh matter tuber yields (t ha-1) as affected by (a) fertilization and (b) cultivar during 2002, 2003 and 2004 (means ± standard error)



In Exp. 2, cv. Marlen in 2003 had higher tuber FM yields than cv. Agria in most cases at the two early harvests, while Agria gave higher yields at the later harvests. In 2004, the positive response of tuber FM yield to pre-sprouting (PRS) lasted throughout the season, but could not be established at final harvest in 2003 (Table 1).

		2003			2004		
cv.	PRS	15 Jul	28 Jul	17 Sep	28 Jul	13 Aug	9 Sep
Agria	yes	22.6	31.5	39.9	29.9	31.1	30.0
	no	19.6	28.2	39.0	26.6	27.6	28.1
Marlen	yes	23.9	32.5	36.1	27.8	29.8	30.7
	no	21.5	30.7	35.2	25.6	27.4	27.4
S. E. of mean		0.53			0.59		

Table 1: Fresh matter tuber yields (t ha^{-1}) as affected by pre-sprouting for two cultivars at subsequent harvests during 2003 and 2004

At harvest in September 2004, though, yield increase by pre-sprouting still amounted to +2.6 t ha^{-1} (+2.8 t ha^{-1} at the end of July) (Table 1). A similar response to pre-sprouting depending on year and date of harvest was established for size-graded (marketable) yields (data not shown). Dry matter (DM) concentration of tubers was significantly affected by fertilization (Exp. 1; Table 2a), cultivar (Exps 1-3) and pre-sprouting of tubers (Exp. 2; Table 2b). Moreover, significant interactions of these treatments with the year were established. Storage increased DM concentration significantly (Exp. 1; Table 2c), in two of three experiments.

a) Fertilizer type	Agria	Marlen	(b)	Yes	No
CM	21.2	22.5	2003	27.6	27.5
PS	21.2	22.7	2004	25.6	24.1
PSHG	20.1	22.0	S.E. of mean	0.11	
HG	20.6	23.0	(c)	At harvest	After storage
CON	21.9	23.5		21.7	22.1
S.E. of mean	0.17		S.E. of mean	0.08	

Table 2: Tuber dry matter concentration (%) as affected by (a) fertilization and cultivar, (b) pre-sprouting and year and (c) storage (means ± standard error)

No effect of fertilization (Exp. 1) and no consistent effect of preceding crop or pre-sprouting (Exp. 2) on reducing sugar concentration (RSC) could be established, but significant (p<0.0001) interactions for storage*year were found in all experiments.

		2003		2004	
Exp	cv.	At harvest	After storage	At harvest	After storage
1	Marlen	6 (70.5)	16 (70.3)	15 (71.6)	66 (64.2)
2		10 (69.6)	17 (70.1)	27 (70.6)	104 (65.8)
3		1 (70.5)	2 (70.5)	2 (71.0)	15 (62.8)
3	Carmona	2 (70.1)	24 (62.8)	6 (69.1)	64 (50.8)
3	Delikat	4 (67.7)	44 (58.1)	18 (62.7)	113 (44.7)
3	Saturna	1 (69.3)	4 (69.2)	5 (71.2)	19 (62.2)
1	Agria	5 (*3.9*)	10 (*3.8*)	15 (*3.9*)	70 (*3.5*)
2		16 (*4.4*)	28 (*4.0*)	29 (*3.9*)	136 (*3.5*)
3		1 (*4.5*)	3 (*3.8*)	2 (*4.1*)	21 (*3.5*)
3	Premiere	11 (*4.1*)	56 (*2.8*)	23 (*3.0*)	90 (*2.2*)
3	Velox	6 (*3.9*)	39 (*2.6*)	21 (*3.3*)	102 (*2.6*)
3	Camilla	2 (*3.6*)	10 (*3.8*)	11 (*2.9*)	72 (*2.4*)
3	Freya	2 (*4.1*)	3 (*3.6*)	3 (*3.8*)	24 (*3.2*)
3	Marena	2 (*4.1*)	3 (*4.0*)	2 (*3.7*)	11 (*3.5*)

Table 3: Tuber reducing sugar concentrations (g kg-1 FW), crisp L-values and French fry quality scores (in italics), respectively (both in brackets) as affected by storage and cultivar during 2003 and 2004 (means ± standard error)

For the 2003 crop, RSC increased during storage (Exp. 1-3), but was still very low after four months of storage (8°C). In 2004, the RSC at harvest was comparatively higher than in 2003, and increased during storage appreciably (Table 3). The response of French fry quality score (mean of weighted characteristics colour [2x], texture [3x] and taste/odour [5x]) was mostly affected by storage (Exp. 1-3) and, additionally, interactions for cultivar*storage (Exp. 3) occurred. For crisp quality, significant interactions for storage*year (p<0.0001) were established. Lighter crisps (higher L-values) were assessed after winter wheat than after the two leguminous crops (data not shown), while after oats L-values were lower than after grass-clover.

Discussion

The positive response of tuber FM yield to cattle manure application in one of three years could be traced back to the increased supply of K, and – possibly a more balanced nutrition with regard to N and K. This suggestion is further strengthened by the profound effect of combined of K and the organic N source (PSHG) on FM tuber yield compared with sole application of N (HG) and K (PS) (Fig. 1a) (Herlihy and Carroll, 1969). Results from Exps 1 and 2 show clearly, that performance of cultivars may vary considerably between the years (Fig. 1b) and seems to depend on the length of the growing season which - in organic potato cropping – is often shortened by late blight (*Phytophthora infestans*) (Finckh et al 2006; Möller et al. 2007). Pre-sprouting could be shown to promote early tuber yield formation and DM accumulation and thereby reduce the risk induced by *P. infestans*, like in 2004 (Table 1 and 2b). Results show that tubers from organic potato cropping may be expected to have sufficiently high tuber DM concentrations for processing into French fries (>19%). However, DM concentrations of tubers may fall short of the minimum of 22% required for crisps when a combined N and K fertilizer is applied. Results give evidence that the development of reducing sugars cannot necessarily be foreseen from the initial reducing sugar level at harvest. Moreover, sugar accumulation during storage seems to be mainly cultivar-specific. The marked increase due to storage for very early and early cultivars suggests that reducing sugars accumulation may strongly depend on maturity type. Throughout the experiments, results confirmed that the individual growing season has a tremendous impact on both, the initial level of reducing sugras and the increase of reducing sugars during storage. The medium-early cv. Agria and medium-late Marena proved to be well suited for cultivation of organic potatoes for French fries. Even in 2004, the season with marked quality losses due to storage, the quality score did not fall below the threshold of 3.5 (Table 3). There was no consistent response of crisp colour to an increased N supply brought forth by leguminous preceding crops or fertilization with horn grits. Even though L-values were significantly reduced by grass clover and peas, preceding crops do not seem to have any relevance to marketability of crisps. Overall, the results indicate that final product quality is much more influenced by growing season, storage and cultivar than by fertilization, preceding crop, or pre-sprouting.

Acknowledgments

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DEVELOPMENTS IN ORGANIC POTATO BREEDING IN THE NETHERLANDS

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Organic growers in the Netherlands are using conventionally bred varieties. Marketable yields were on average around 24 ton per hectare, where conventional growers harvest 55 tons per hectare. This was caused by the limited growing season due to the late blight (*Phytophthora infestans*) attacks in July. And also because of the high percentage of tare, due to problems with *Rhizoctonia solani*. Farmers started asking for better adapted varieties with an average yield of 30 tons per hectare.

In 1988, the acreage of organic potatoes was rapidly expanding and the first trials to find these better adapted varieties were planted. For these trials, existing varieties were chosen with good tuber blight resistance and an acceptable combination of earliness and foliage blight resistance. These varieties were tested for adaptability to low input conditions in organic fields. Major selection characteristics were yield, tuber appearance and resistance to late blight and tolerance to *Rhizoctonia*. The first successes were achieved with varieties with known late blight resistance genes (R-genes) like the cultivars Escort and Raja.

Testing these existing commercial varieties under organic conditions, it became clear soon that with the increased virulence of late blight, varieties with better resistances were necessary. In the selection program, breeding clones were selected with acceptable earliness and late blight resistance and incorporated in trials on organic farms. Further, in the pre-breeding programs for late blight resistance, the most promising clones were selected and tested on organic fields for adaptability and field resistance to late blight under natural disease pressure.

Major selection criteria at that time were foliage resistance to late blight, yield potential under organic conditions and some cooking quality. The first results of this screening were rejected soon after market introduction began. It turned out that the rapid developments in the market, demanded that organic potatoes look at least as good as conventional grown potatoes.

Recent developments, with very early infections of late blight and yields that have dropped to 10-15 tons per hectare have forced the breeding of varieties for organic farming towards the use of absolute resistances to late blight or very early tuber set in combination with earliness. The first results of these efforts led to the variety Toluca that entered in the Dutch national variety list in 2007.
Variety Toluca carries a single R-gene from *Solanum bulbocastanum* and responds to a late blight attack with clear visible necrosis of the leaves. The potential yield of this variety is rather low, but the stability of yield in "blight years" is much better than average and the variety looks acceptable for the pre packing industry.

In resent years Agrico Research, but also the Laboratory for Plant Breeding from the University of Wageningen have put great effort into finding more major resistance genes in wild potato species and these now have to be brought to an acceptable agricultural level. The results of these efforts, in combination with the intensive screening of the best clones on three organic fields in the Netherlands and six organic fields in other European countries will become visible in the near future.

Also, organic growers were stimulated to start a small potato breeding program on their organic farms. Now three organic hobby breeders are active in the Netherlands and a program is started by the Louis Bolk Institute to stimulate more organic growers to start breeding potatoes and to supply them with good genetic material. The first variety of one of these hobby breeders is in second year trials for the Dutch national list.

CULTIVATION AND ANALYSIS OF ANTHOCYANIN CONTAINING TYPES OF POTATOES IN ORGANIC AND INTEGRATED FARMING SYTEMS REGARDING CULTIVABILITY AND ADDITIONAL HEALTH BENEFITS

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Abstract

In a two year research project a representative spectrum of blue potato varieties were cultivated and tested in detail regarding disease infestation, yield potential and the influence of production systems (organic/integrated). Cultivation recommendations for blue potatoes could be deduced from this. Furthermore the varying anthocyanin content as well as the antioxidant capacity of the types used were analysed. Types with a particularly high content will undergo further tests to show the influence of the manner of preparation (boiling, steaming, frying) and determine their use for the processing industry. The cultivation of high yield blue types can be an alternative to the cultivation of yellow fleshed high yield types in organic or integrated operating farm companies.

Introduction

Anthocyanin, the phytochemical which appears in various useful plants such as potatoes, cereal and vegetables, known especially for its health promoting effects in red wine, have a health promoting effect with their antioxidant properties (KATSUBE et al. 2003, KÄHKÖNEN 2003, MURCOVIC 2002, WATZL et al. 2002). The health promoting properties of anthocyanin are determined by its antioxidant capacity. The health promoting features are for example protection against DNA damage, degenerative illnesses and boosting the immune system (WEISEL 2006). As the potato with a consumption of 63 kg/person/year in Germany (ANONYMOUS 2007) still has an important position as a basic food, it is increasingly in the interest of the consumer, nutritional medicine and the food processing industry (e.g. potato crisps production). The goal of the interdisciplinary AGIP research project is the compiling and evaluation of the influence of the production system with various intensities (fertilisation/no fertilisation) as well as kitchen preparation techniques on the anthocyanin content and the antioxidant capacity of selected culture plants.

Materials and methods

Within the project a field test was carried out in Germany at the Waldhof (organic farmed) and Nettehof (integrated farmed) experimental station at the FH [University of Applied Sciences] Osnabrück. In the test a compilation and evaluation of the influence of various cultivation parameters on technically more favourable type features with 11 blue flesh types of potato took place. The manner of storage and preparation of foods has an influence on the anthocyane content and antioxidant capacity on the ready to consume end product, which will be analysed in the course of the project. To document the effect of storage, there are various time frames for analysis: a) directly after harvesting: investigations into raw harvest crop = analysis of original content b) after 8 weeks of storage: harvest crops = determination of storage losses (raw), c) after 16 weeks of storage: = determination of storage losses (raw), determination of preparation losses. The effect of preparation (e.g. boiling, steaming) on the anthocyane content will be quantatively and qualitatively analysed in 2008 and evaluated in co-operation with the TU [Technical University] Braunschweig using the HPLC method (HILLEBRAND 2004). The determination of the antioxidant capacity was carried out using the TEAC test at the FH Osnabrück (HILLEBRAND 2004). In the course of the project a test tasting will be carried out according to predefined standards (hedonic test BUSCH-STOCKFISCH 2004). In this way a taste cognition will be evaluated by trained and untrained test eaters (test persons).

Results

The selected types of potato display significant differences in yield between the varieties in cultivation year 2006 and 2007, in organic and integrated cultivation procedures (Tab. 1). Vitelotte and Red Cardinal (2006)/Highland Burgundy Red (2007) only showed a very small yield in both cultivation years. Other types such as Blue Salad Potato or Olivia had higher yields in 2007 than in 2006.

Tab. 1: Yields of selected types of blue potatoes in 2006 and 2007 in organic and integrated farming

	Total yield (t ha^{-1}) (organic cultivation) 2006	Total yield (t ha^{-1}) (organic cultivation) 2007	Total yield (t ha^{-1}) (integrated cultivation) 2006	Total yield (t ha^{-1}) (integrated cultivation) 2007
Vitelotte	2,0 (a)	2,9 (a)	2,6 (a)	13,7 (a)
Red Cardinal (2006)/Highland Burgundy Red (2007)	2,9 (a)	4,0 (a)	4,1 (b)	20,5 (ac)
Olivia	7,3 (b)	18,4 (b)	11,9 (c)	54,5 (b)
Blue Salad Potato	4,4 (c)	11,79 (c)	7,8 (d)	33,0 (c)
Organic cultivation: 2006 SD: LSD 1,15; 2007 SD: LSD 5,93 Integrated cultivation: 2006 SD: LSD 0,76; 2007 SD: LSD 14,83				

Fig. 1 shows the anthocyanin content of selected types of blue potatoes (organic cultivation) after four, eight and sixteen weeks of storage: the anthocyanin content of Red Cardinals is partly increasing, but in Olivias and Blue Salad Potatoes it is decreasing. Fig. 2 shows the results of the integrated farming system and gives the same tendency. Results of the variety Vitelotte in 2006 could be determined after four weeks. Because of less material tests after eight and sixteen weeks could not be done.

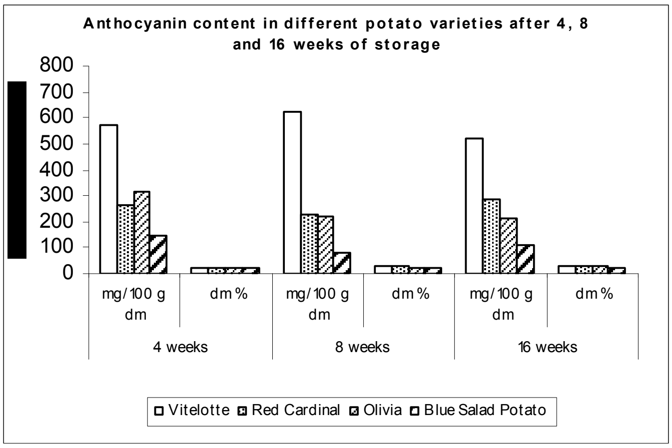

Fig. 1: Anthocyanin content in mg/100g dry matter of different potato varieties after 4, 8 and 16 weeks of storage in 2006 (organic cultivation)

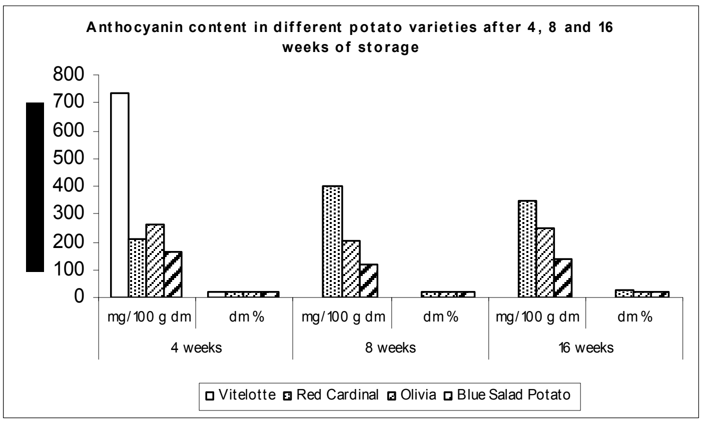

Fig. 2: Anthocyanin content in mg/100g dry matter of different potato varieties after 4, 8 and 16 weeks of storage in 2006 (integrated cultivation)

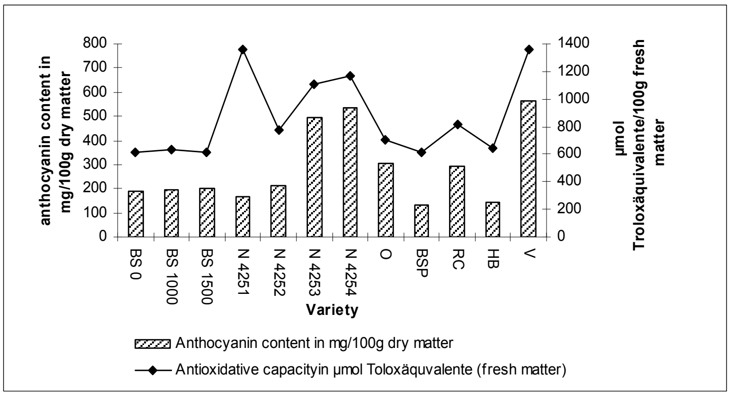

Fig. 3: Relation between anthocyanin content and antioxidative capacity of different blue potato varieties 2006 (organic cultivation) Abbreviations: BS 0 = Blauer Schwede, without fertilization; BS 1000 = Blauer Schwede, 70 kg/N/ha; BS 1500 = 105 kg/N/ha; N4251 – N 4254 = Norika breeding strains; O = Olivia; BSP = Blue Salad Potato; RC = Red Cardinal; HB = Herrmanns Blaue; V = Vitelotte

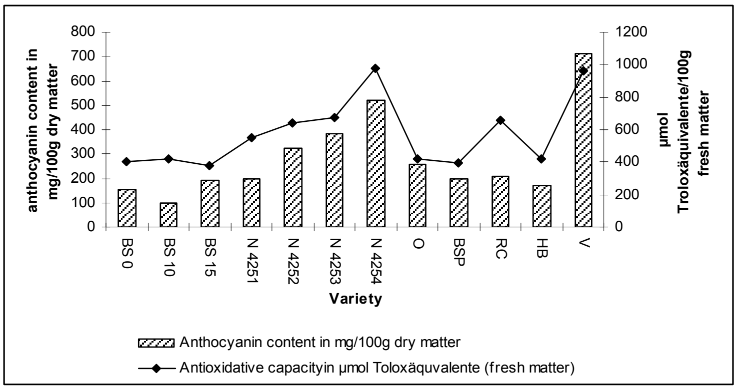

Fig. 4: Relation between anthocyanin content and antioxidative capacity of different blue potato varieties 2006 (integrated cultivation) Abbreviations: BS 0 = Blauer Schwede, without fertilization; BS 1000 = Blauer Schwede, 70 kg/N/ha; BS 1500 = 105 kg/N/ha; N4251 – N 4254 = Norika breeding strains; O = Olivia; BSP = Blue Salad Potato; RC = Red Cardinal; HB = Herrmanns Blaue; V = Vitelotte

Fig. 3 and 4 illustrates the antioxidant capacity of the cultivated types in relation to the anthocyanin content at organic and integrated cultivation. The investigations in 2006 showed that the level of anthocyanin influenced the antioxidant capacity and therefore the health promoting effects.

Discussion

The year 2006 was characterised by adverse climate conditions. Strong rainfall in April prevented optimum planting of the potatoes. The high temperatures and missing rainfall in June had a negative effect on the tuber growth. Layers and second shoots formed. As a result the exterior and interior quality of the potatoes was often not satisfactory (infestation with potato scurf (*Streptomyces scabies*)). The yields achieved could not be regarded as representative. In the investigations of 2007 it could be recognised that all blue potato varieties showed better results in yield by integrated cultivation. Vitelotte, Highland Burgundy Red and Red Cardinal could not achieve an adequate yield in organic cultivation. Other types showed good harvest crop yields up to a total yield of e.g. 54,5 t ha^{-1} in integrated cultivation with Olivia.

Vitelotte showed the highest anthocyanin content but is inapplicable for cultivation because of the low yield and its skin texture. Basic causes for the higher yield under the integrated cultivation system depends on better possibility of plant protection especially against *Phytophthora infestans*.

The investigations in 2006 showed that the anthocyanin content has an influence on the antioxidant capacity and therefore on the health promoting effects. Anthocyanin content and antioxidant capacity are dependent on type and according to the results from 2006 are not influenced by the use of a nitrogen fertiliser or the cultivation system (organic/integrated). The results regarding to anthocyanin content and antioxidative capacity showed no increasing or decreasing effect concerning to the different cultivation systems. Differences in the level of both values depend on the potato varieties and/or on prevailing stressors like heat and aridity.

Some of the tested varieties showed disadvantageous concerning to skin texture, shape or taste. Especially Red Cardinal and Vitelotte showed an unfavorable skin texture with deep eyes. These varieties can only be used as potatoes with skin because the peeling losses would be too high.

Conclusions

Blue potatoes are cultivated only in very small quantities in Germany at the moment, but varieties and yield are not documented. The results of the research project show that it is possible to cultivate particular type's e.g. old anthocyanin containing types of potatoes in organic and integrated farming; however a breeding process must take place concerning the needs of the user today regarding skin texture, shape or taste. The additional health benefits of food are brought more and more to the attention of the public. In addition a positive influence on biodiversity can be expected in the agricultural sector.

Acknowledgments

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Physiology

YIELD AND BIOMASS PARTITIONING IN EXTRASEASONAL POTATO CYCLES IN SICILY

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INTRODUCTION

In recent years, the Italian extraseasonal potato cultivation including crops realised in the winter-spring cycle and in summer-autumn cycle (namely "novella" and "bisestile", respectively) covered a land area of about 20,000 ha (Istat). The Italian regions concerned with these crops are primarily southern regions. From planting to harvest, the climatic conditions such as temperature, photoperiod and solar radiation which the plants encountered in the two extraseasonal cycles have a very different trend and can have an appreciable effect on cycle length, tuber differentiation and biomass partitioning compared to those cultivated in the spring-summer cycle (Mauromicale et al., 2003). In particular, the winter-spring cycle which runs from November-December to May, allows the plants to take advantage of climatic conditions (short photoperiod and relatively low temperatures) that aid tuber production, while the summer-autumn cycle from August to December, at least in the first stage, may mean unfavourable conditions for the start and growth of tubers. The effect of these constraints is enhanced by the use of foreign varieties bred outside and selected for different climatic conditions. This prompted a research project on "Potato breeding", funded by the Italian Ministry of Agricultural and Forestry Policies from 1997 to 2005, which among other objectives had that of constituting new varieties for extraseasonal cycles (Frusciante et al., 1999). Many of the new Italian genotypes have been evaluated both in the winter-spring as well as summer-autumn cycles (Ierna et al., 2005 a and b; Parisi et al., 2002; Ranalli et al., 1997; Tedone et al., 2005), but until now a systematic comparison of potato genotypes between the two cycles has been lacking. The aim of this research was to compare biomass partitioning and yield in four genotypes grown both in winter-spring and summer-autumn cycles.

MATERIALS AND METHODS

Trials were carried out in 2000 along the coastal plain south of Siracusa, Italy (37° 03' N, 15° 18' E, 10 m a.s.l.) which is a typical area for extraseasonal cycle potato cultivation in Sicily. The soil type is calcixerollic xerochrepts (USDA Soil Taxonomy), moderately deep, with a loam-clay texture.
In 2 extraseasonal crop cycles (winter-spring and summer-autumn) 4 genotypes (Spunta, Sieglinde, Daytona and Ninfa) of potato (*Solanum tuberosum* L.) were studied. Plantings with whole seed tubers were made on 5 January in the winter-spring cycle and on 26 August in the summer-autumn cycle.
In each extraseasonal crop cycle a randomised block design with three replications was used. Plot size was 3.0 by 2.8 m, with plants spaced 0.3 m apart, in rows separated from one another by 0.7 m (equivalent to a plant density of 4.76 plants m^{-2}).

The two cultivars Spunta and Sieglinde are the most widely cultivated in the Mediterranean region. Spunta is an early ripening ware potato, with long, regular and very large tubers; plants produce few erect and vigorous stems. Sieglinde is a firm flesh, early cultivar with oblong, regular and moderate-sized tubers; plants produce numerous stems which are of medium height, semi-erect and moderately vigorous. Daytona is a new Italian variety, of late maturity with short, oval and regular tubers; stems are of medium size. Ninfa is also a new Italian variety, late maturing, with oblong, regular and very large tubers; stems are robust and erect (Ranalli et al. 2005).

The usual crop management was used.

Plants were harvested by hand at 120 (winter-spring cycle) and 105 (summer-autumn cycle) days after planting, when about 70% of haulm was dry. Plants were individually separated into stems, leaves, roots and tubers, and weighed. The tubers of each plot were counted and weighed. Samples of

all plant parts were then dried at 80 °C and weighed.
Meteorological data were also collected.

All data were submitted to Bartlett test for the homogeneity of variance and then analysed using ANOVA as a factorial combination of crop cycle x genotype. Means were compared by *LSD* test, provided the *F* test was significant. CoStat version 6.003 was utilized.

RESULTS

The mean maximum and minimum temperatures and photoperiod during cropping seasons are shown in Figure 1.

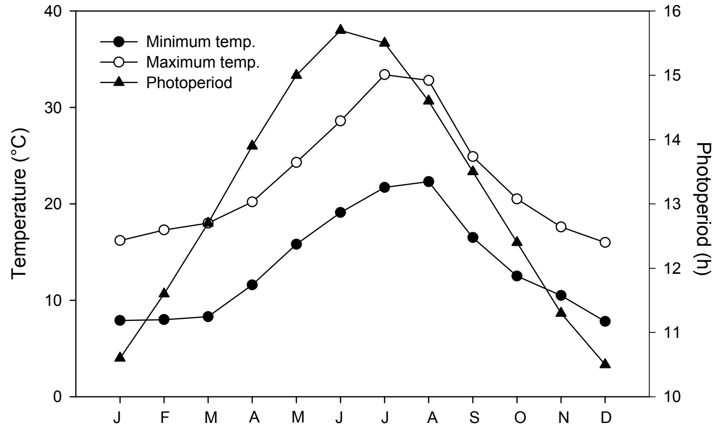


Fig. 1 - The mean monthly maximum and minimum air temperatures and photoperiod during the growing seasons.

Source/sink ratio

The source/sink ratio which is the ratio between dry weight of leaves and dry weight of tubers, was influenced significantly by crop cycle, genotype and their interaction. On average of genotypes, the ratio was significantly higher in summer-autumn cycle than the winter-spring cycle (0.255 vs 0.180). On average of crop cycle, Spunta showed the highest value (0.275), Daytona and Sieglinde with negligible difference the lowest (0.181 and 0.188, respectively), and Ninfa intermediate (0.225) (Fig. 2).

From winter-spring to summer-autumn cycle the value remained unvaried in Daytona, while it significantly increased in Spunta (+114%), Sieglinde (+39%) and Ninfa (+25%) (Fig. 2).

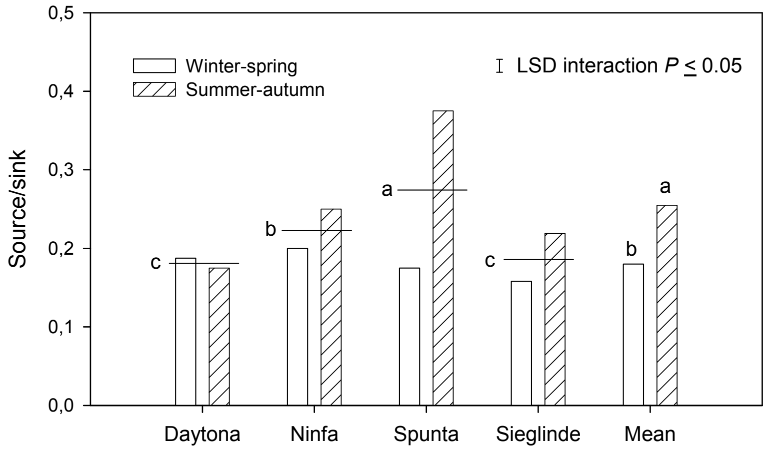


Fig. 2 - Source-sink ratio in relation to crop cycle and genotype.

Yield and components

Irrespective of the crop cycle Ninfa proved the most productive (34.5 t ha^{-1}), followed by Daytona (28.5 t ha^{-1}), then Spunta (25.5 t ha^{-1}) and Sieglinde (20.5 t ha^{-1}) (Fig. 3).
All the genotypes produced greater yields in the winter-spring than in the summer-autumn cycle (on average 33.0 against 21.5 t ha^{-1}). However, the differences in yield were very high in Ninfa (154%), and much lower in Daytona (28%) and in Spunta and Sieglinde (22%).

In the winter-spring cycle, Ninfa was easily the most productive with almost 50 t ha^{-1}, followed by Daytona with 32 t ha^{-1}. This latter, together with Spunta, were the most productive in the summer-autumn cycle (25.0 and 23.0 t ha^{-1}, respectively).

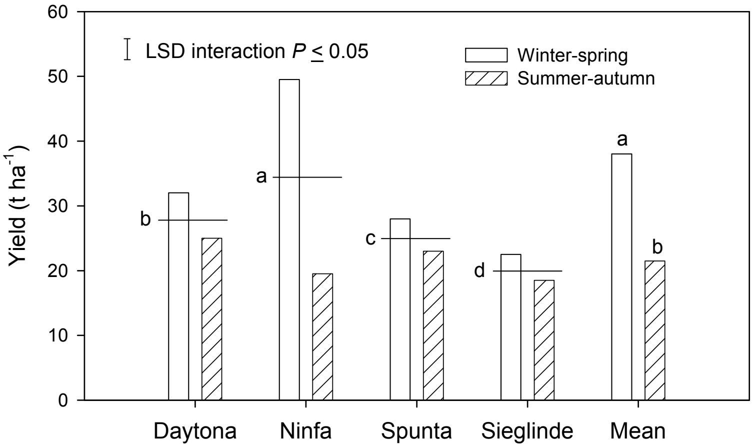
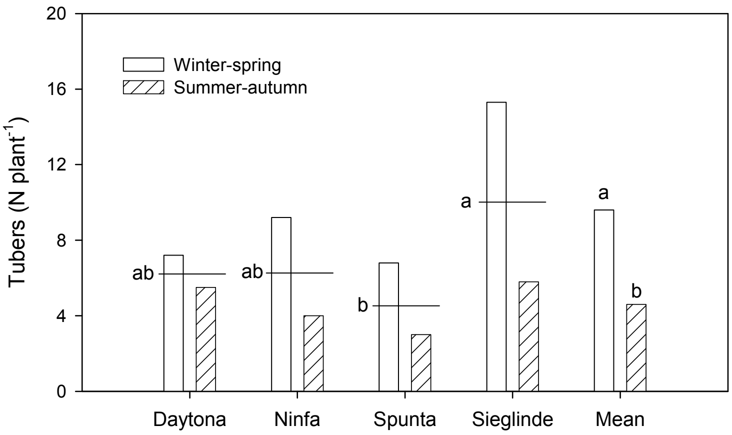


Fig. 3 - Tuber yield in relation to crop cycle and genotype.

The greater production of the plants grown in the winter-spring rather than the summer-autumn cycle was determined by a larger number of tubers per plant (9.6 against 4.6, on average of genotypes) (Fig. 4). The average unit weight of the tubers was, instead, higher in the summer-autumn rather than the winter-spring cycle (112 against 79 g). The differences in the unit weight of the tubers in relation to the growing cycle were significant only in Spunta and Sieglinde, in which, passing from the winter-spring to the summer-autumn cycle the average unit weight more or less doubled (Fig. 4).



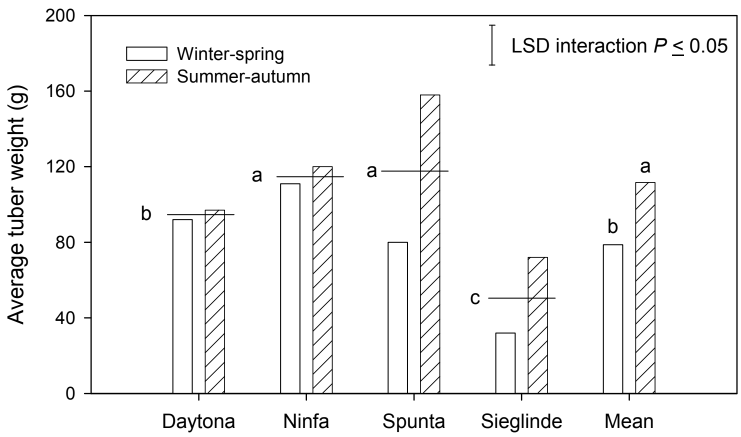


Fig. 4 - Number of tubers (top) and average tuber weight (bottom) in relation to crop cycle and genotype.

DISCUSSION AND CONCLUSIONS

The data presented in this study reflect the importance of evaluating the potato genotypes at the same time in the two extra-seasonal cycles. The crops in the winter-spring with respect to the summer-autumn gave higher tuber yield and lower source/sink ratio values, which would appear to indicate a good efficancy in the translocation of the photosynthetates from the sites of production (leaves) to those of accumulation (tubers). The genotypes showed, however, different productive responses in relation to the crop cycle depending largely on their capacity to compensate the lower number of tubers per plant in the summer-autumn cycle, with a greater weight of the same tubers. This capacity fairly low in Ninfa, which explains the major yield differences of this genotype in the two cycles, proved very high in Spunta and Sieglinde. It seems that these two varieties have self-regulating mechanisms which allow reaching a state of tuber yield equilibrium also when conditions are not very advantageous for tuber production and growth. These differential responses of the genotypes suggest a strategy for the improvement of adapting consolidated as well as new emerging varieties to extraseasonal cycles.

ACKNOWLEDGEMENTS

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PHYSIOLOGIC SUPPORT OF EARLY GROWN POTATO HARVEST MANAGEMENT

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The process of management of potato tuber harvesting can be divided into several stages. To solve the problem of management of early grown potato productivity the crop biology should be studied carefully. The process of potato plant development abroad include 9 stages as far as in Russia the same process is divided into 5 periods and 12 organogenesis stages. Here is the description of each organogenesis stage. The 1st stage: at this stage there are first simple leaf rudiments in the knobbing cone and the plant is in the state of physiological dormancy (see picture 1). The 2nd stage: a stage of plant root development. The same stage for early varieties is characterized by stolon formation. As for knobbing cone, rudimentary compound leaves are formed here. The 3d stage of organogenesis: it is marked by knobbing cone differentiation and a stolon forming in early varieties. The 4th stage: The flower tubercles of future blossom clusters are laid at this stage. The 5th stage: it is the stage of corolla formation and the period when a stamen tubercle and a pistil are laid. Here the heavy stolon development goes on.

The 6th stage: both male and female gametophytes are formed here. The 7th stage: stamens and pistils are well developed and covered by corolla. The flourishing shoots of the main stem blossom clusters are stretching. The tuber forming begins. The 8th stage: the petal steps out of the calyx limits. Intensive forming of new tubers and growth of the formed ones takes place at this stage. Forming of generative propagation potato organs coincides with forming of vegetative propagation potato organs and these two processes are closely connected. Thus florescence and tuberization do not compete in usage of plastic matters forming during photosynthesis. The above mentioned allows to mark 5 periods of potato plant development. The period when tuber sprouting begins till the time of emergence is considered to be the first one. Nowadays the relation between row width and root system spreading of potato itself along soil profile in different agro-technologies is not considerable. The row width now is determined by energy value of the mover, by the truck width and by the whole machinery complex used for crop production.

Our researches revealed that value of early grown potato root system used in soil value changes and depends on variety peculiarities and on the moisture rate of the soil layer taken into consideration during irrigation. The second stage of potato productive processes management requires involving both physiological and biochemical, agro-climatic, economic and managing indices. The influence degree of each factor affecting the production process is not constant. Normally potato plant reactions depend on the number of internal and external growth conditions.

In getting high early potato yields special attention is paid not only to the role of varieties but also to the speed of assimilation apparatus, to the size of the active leaf surface, to the life-time of healthy leaves.

The programming of early potato tuber production consists of a number of steps: agro-technical, organizational, economic etc. These steps made timely and in proper way will result in high crop capacity from both economic and energetic point of view.

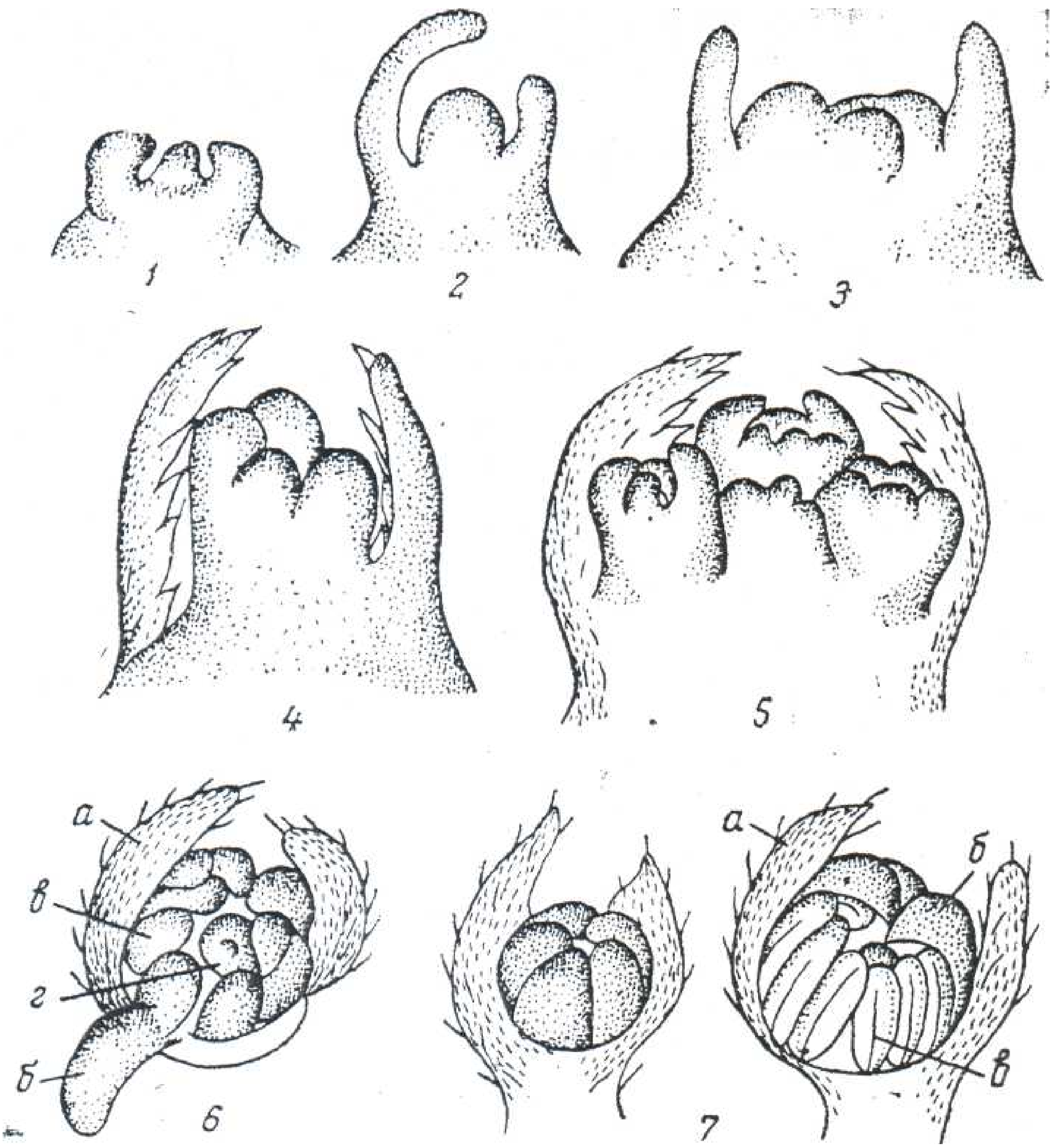
Here are the following crop capacity levels and categories to be marked.

1st level. Farm crop capacity. The planting productivity of this category is received from a specific part of crop rotation field while involved in farm production.

2d level. Programming crop capacity. This category embraces the planting productivity with application of main agro-technical steps according to the technological cultivation and harvesting schedule of potato. The factors limiting the realization of this crop capacity degree are the achieved farming culture level, the level of agro-biological, material technical and financial resources support as well as skills to estimate and use current information and forecasts. Planned crop capacity is realized by using different traditional domestic and foreign machinery agro-technologies.

3d level. Actually possible crop capacity. The planting productivity here is achieved by using the complex of agro-technologies applied under actual meteorological conditions on the experimental plot. Soil fertility indices are factors limiting realization of this crop capacity level. The actually possible crop capacity is realized by the use of different machinery technologies of biological and adaptive plant-growing.

4th level. Crop capacity supported by climatic conditions. This category explains the crop capacity productivity which can be achieved under ideal soil climatic conditions as well as under actual meteorological conditions when accomplishing all the agro-technological complex. Limiting factors for this crop capacity kevel are planting warm and moisture support indices. Crop capacity supported by climatic conditions is realized by the use of different integral agro-technologies in the integrated plant-growing complex.



Notes to the pictures:
Picture 1. Potato organogenesis stages.
1 - I; 2 - II; 3 - III; 4 - IV; 5 - V; 6 - VI
Forming of generative flower parts – stamens and a pistil: a – sepal; b – crown petals; c – stamen; d – pistil; 7 – VII. Flower parts and blossom cluster development.

5th level. Potential crop capacity. At this level the crop capacity productivity can be achieved under soil and meteorological conditions while accomplishing all the agro-technological measures. The limiting factors of this crop capacity level are active photosynthetic radiation and agro-biological possibilities of modern crop varieties and hybrids. By the use of different space and differentiated agro-technologies potential crop capacity is realized in the system of exact plant-growing.

6th level. Biological productivity of individual plants, i.e. theoretically possible yield while studying productive process regularity during all the life period of plants and optimum plant need support in each period of plant life. The limit of actual daily photosynthesis efficiency is 20%, those of breath is 8% of active photosynthetic radiation absorbed.

It is clear now that all crop capacity levels correspond to any basic agro-technologies and to the level of plant-growing development. Moreover they are of accidental character, changing from year to year. This circumstance is particularly important both for science and for potato-growing practice.

Such a phenomena shows the unity as well as the conflict of two opposites, i.e. crop capacity increase and its stability over the years. When the coefficient of crop capacity variations decreases on the way of innovative plant-growing development this results in gradual removal of the conflict.

The actual crop capacity for the coming year is impossible to determine that's why crop capacity programming is a task of probability. While solving this task both crop capacity constant and its achievement probability should be calculated. For example to get proper estimation of soil fertility level the average quadratic deflection, the variation factor and other statistic constants of separate analysis indices of soil samples should be known.

The theory base developed by authors of integral and space differential agro-technologies of early grown potato is the study of stable productive process regularities and their application in agro-biosynthesis. These regularities are conditioned by interaction of soil and plant as biological organisms. The early grown potato productivity means the capacity of potato plant to give maximum output of dry matter from a unit of area, used by the concrete summer harvesting time. Dry matters of potato are starch, protein, fats, vitamins, mineral salts. These components make up the most valuable part of new tuber harvesting.

Productivity being the system and complex feature of early grown potato is the subject to many researches. But domestic as well as foreign farmers used to consider it separately from growing conditions and without taking in account the interaction between different productivity components during potato plant development and growth.

The change of any parameters may result to yield increasing if optimal leaf surface of planting will be maintained. High productive plantings are produced on optimal correlation of architechtonique indices of individual plants and planting in general. In order to characterize the duration of photosynthetic work of plantings we use the index of photosynthetic potential (PhSP), which is in close positive correlation with tuber yields.

Table 1. The effect of agrocomplex on bio-morphologic indices of early grown potato variety "Iskra".

Tuber size, g	Stand thickness, amount/ha	Number of stalks, amount/ha			Number of tubers per a stalk		
		Planting terms					
		1	2	3	1	2	3
40	40	138	157	140	5,1	5,0	5,3
	50	178	196	171	4,8	4,8	5,1
	60	211	233	209	4,6	4,5	4,6
	70	244	274	241	4,3	4,3	4,5
70	40	164	190	173	5,0	5,1	5,1
	50	210	241	212	4,6	4,8	4,8
	60	244	280	259	4,3	4,6	4,4
	70	287	336	296	4,1	4,4	4,2
90	40	200	209	193	4,5	5,4	5,0
	50	245	263	243	4,2	5,0	4,6
	60	291	314	285	3,9	4,5	4,2
	70	335	361	321	3,6	4,1	4,0

The closer to the optimum leaf area (60-70 thousand m²/ha) the stronger this correlation. The plantings are considered to be good if PhSP is not less than 2 mln m² ·day/ hectare, taking into account 100days of actual vegetation. Optimum growth of leaf surface and formation of high PhSP can be supplied with proper agritechnique.

Experiences of 1990-1995 were carried out to study optimal planting parameters. They helped to reveal important regularities, shown on the tables 1, 2 and 3 below.

The best results in dry biomass accumulation were achieved during the years of favorable meteorological conditions of vegetative periods. So in 1990 the level of dry biomass hesitated from 10.7 to 22.7 t/ha. During arid periods dry matter biomass didn't exceed 8.8…16.6 t/ha.

Having studied the patterns of the second period planting the best rate of starch accumulation was with the stand thickness of 60-70 thousand tubers per hectare. The maximum amount of starch was with the stand thickness of 50 thousand tubers per hectare.

Table 2. The effect of agrocomplex on the leaf functioning of early grown potato variety "Iskra".

Tuber size, g	Stand thickness, amount/ha	Leaf square, thousand m²/ha			Photosynthetic potential, mln.m²·days/ha		
		Planting terms					
		1	2	3	1	2	3
40	40	40,0	49,2	48,3	1,6	1,8	1,7
	50	44,6	53,5	52,8	1,8	2,0	1,9
	60	44,4	52,5	52,7	1,8	2,0	1,9
	70	43,6	51,2	51,2	1,8	2,0	2,0
70	40	54,5	65,4	62,4	2,2	2,5	2,3
	50	57,0	67,8	65,6	2,4	2,6	2,4
	60	55,8	64,8	62,8	2,3	2,5	2,4
	70	52,0	60,2	58,1	2,2	2,4	2,3
90	40	60,0	71,0	65,7	2,5	2,7	2,5
	50	60,6	71,8	67,7	2,6	2,8	2,6
	60	58,2	66,5	64,4	2,5	2,6	2,5
	70	52,8	61,2	59,2	2,4	2,5	2,4

Table 3. The agrocomplex effect on the productivity of early potato grown variety "Iskra".

Tuber size, g	Stand thickness, amount/ha	Pure photosynthesis productivity, g/m²·days			Pure crop capacity, t/ha		
		Planting terms					
		1	2	3	1	2	3
40	40	5,4	5,9	5,8	24,7	29,2	27,0
	50	5,7	6,1	6,0	28,0	32,7	30,1
	60	5,7	6,1	6,0	27,3	31,5	29,2
	70	5,7	6,0	6,0	26,3	30,0	28,0
70	40	5,9	6,1	6,0	35,4	40,7	36,7
	50	5,9	6,3	6,2	38,0	42,9	39,3
	60	5,9	6,2	6,1	35,6	39,3	36,1
	70	5,9	6,1	6,1	32,7	35,2	32,7
90	40	6,2	6,4	6,2	41,6	45,7	41,3
	50	6,3	6,5	6,4	43,1	46,7	42,4
	60	6,2	6,4	6,3	38,7	40,5	37,3
	70	6,1	6,4	6,2	35,9	37,2	34,3
LSD_{05},						1,3	

REGULATION OF STEROIDAL GLYCOALKALOIDS IN POTATO

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Steroidal glycoalkaloids (SGAs) are secondary metabolites characterized by a bitter taste and known to be toxic when consumed in large quantities. The two major SGAs present in potato *(Solanum tuberosum)* cultivars are α-chaconine and α-solanine that exhibit strong lytic properties and inhibit acetyl choline-esterase activity. Because of their toxic effect, commercial cultivars contain low level of SGAs, and their content in the edible tuber should not exceed 20mg/100gr FW. However, several factors associated with growth, harvest and post-harvest treatments, such as light exposure and wounding, might lead to an increment in tuber SGA content. Therefore, it is desirable to develop new improved potato genotype that exhibit high SGA level in leaves to protect the crop against insects and fungal diseases, and none in the edible tubers.

The SGA biosynthesis is derived from the isoprenoid pathway, however only sketchy details are available on SGAs specific genes. To understand the regulation of SGA biosynthesis, the expression level of genes involved in the biosynthesis of terpene and sterol derivatives was monitored, by sqRT-PCR, in leaves and tuber skin of potato genotypes that exhibit various levels of SGA content. Results indicated of association between high SGA levels in leaves and tubers and high expression of *hmg1* and p*ss1* genes (Krits et al., 2007). The former encodes for 3-hydroxy-3-methylglutaryl coenzyme A reductase 1 (HMGR1), the committed step of isoprenoid formation by the mevalonate pathway and the latter encodes for squalene synthase 1 (PSS1). Transcripts of other key enzymes of branches of the isoprenoid pathway, vetispiradiene/sesquiterpene synthase (*pvs1*) and sterol C24-methyltransferase type1 (*smt1*), were undetectable or exhibited stable expression regardless of SGA content, respectively, suggesting facilitated precursor flow to the SGA biosynthetic branch. The transcript ratio of solanidine glucosyltransferase (*sgt2*) to solanidine galactosyltransferase (*sgt1*) was correlated to the documented chaconine-to-solanine ratio in the tested genotypes. Significantly higher expression of *hmg1, pss1, smt1, sgt1* and *sgt2* was monitored in the tuber phelloderm than in the parenchyma of the tuber's flesh, targeting the former as the main SGA-producing tissue in the tuber, in agreement with the known high SGA content in the layers directly under the tuber skin.

Sequence comparison of *hmg1* and *pss1* from the cultivar Desiree (low SGA producer) and the wild type *S. chacoense* Bitter (high SGA producer) indicated of genotype specific amino acid substitutions that may affect protein efficiency in metabolism. This result implied that SGA accumulation in the high producers may result from high efficiency of enzymes in the biosynthetic pathway, in addition to the high expression level of the corresponding genes. Accordingly, Desiree plants were transformed with *hmg1* and *pss1* from S. *chacoense* Bitter to induce SGA biosynthetic pathway for identification of genes and intermediates in the modified pathway.

Krits P, Fogelman E, Ginzberg I (2007) Potato steroidal glycoalkaloid levels and the expression of key isoprenoid metabolic genes. Planta 227:143-150.

EFFECT OF CALCIUM AND BORON IN POTATO TUBERS (*SOLANUM TUBEROSUM*) OF VARIOUS CULTIVARS DIFFERING IN BLACKSPOT SUSCEPTIBILITY

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Blackspot is a physiological disorder in potato tubers. The failure reduces the commercial value of the crop depending on percentage of affected tubers (Ozgen *et al.* 2006). It is caused by an impact of the tuber against a hard surface which takes place during harvesting and assorting. As a result of phenolics reaction discoloration occurs 24 to 48 hours after impact. Cells collapse, releasing ethylene, and further cell wall break down leads to cell death, consequently (Pavlista 2005). In this context the physical properties of the cell wall are important. Calcium (Ca) and Boron (B) are involved in the stabilisation of the middle lamella and therefore essential for the maintenance of the cell wall. Ca has a positive effect on the functional integrity of the cell wall and Ca- pectate influences the cell wall plasticity and elongation (Yamauchi *et al.* 1986). A complex of B and hydroxyl groups can stabilize the cytoplasmic membrane and enhance its permeability. B has an influence on the lignin synthesis and lignification of cell walls. Particularly B inhibits the accumulation of phenolic substances which promote lignin degradation. B deficiency in potato tubers induces a concentration of phenolics compounds and a formation of melanin pigments, consequently (Bergmann 1993).
The aim of the present study was to evaluate the concentrations of Ca and B in eight potato cultivars with different blackspot susceptibility. The tubers were grown in sandy soil at the Potato Research Station Dethlingen (VSD) during three vegetation periods (2005, 2006, 2007). Ensuing cold storage (4°C, 95 % relative humidity) for five and eight month affect the blackspot susceptibility of the tubers as well as the concentration of Ca and B. To get comparable results, tubers with a diameter of 40 – 50 mm have been used. Data from the first period are presented in Table 1.

Table 1: Blackspot susceptibility (Index %) in relation to Ca (g kg^{-1} TM), B (mg kg^{-1} TM) and Ca/ B ratio of potato varieties harvested 2005 and stored for five and eight month, respectively

Storage (month)	Variety	Afra	Adretta	Granola	Renate	Nicola	Lolita	Marabel	Gala
0	Index (%)	63.16^a	58.73^a	58.88^a	25.90^b	3.18^c	2.68^c	1.93^c	1.18^c
	Ca (g kg^{-1}TM)	0.47^a	0.51^a	0.46^a	0.46^a	0.40^b	0.36^b	0.41^b	0.39^b
	B (mg kg^{-1}TM)	5.39^b	4.79^b	5.49^b	5.87^b	5.12^b	5.37^b	5.13^b	6.86^a
	Ca/ B ratio	87.94	107.40	83.42	78.41	78.42	67.91	80.00	56.10
5	Index (%)	32.92^b	45.93^a	20.40^b	3.89^c	7.92^c	1.53^d	3.17^c	0.32^d
	Ca (g kg^{-1}TM)	0.32^b	0.39^a	0.39^a	0.41^a	0.32^b	0.41^a	0.42^a	0.36^b
	B (mg kg^{-1}TM)	4.81^b	4.74^b	4.82^b	5.17^b	4.71^b	4.48^b	4.79^b	5.59^a
	Ca/ B ratio	67.45	83.07	80.88	78.86	67.24	90.88	87.87	64.68
8	Index(%)	59.23^b	62.62^a	39.38^b	11.74^c	18.02^c	13.95^c	3.37^c	3.10^c
	Ca (g kg^{-1}TM)	0.47^b	0.63^a	0.65^a	0.63^a	0.56^b	0.60^a	0.63^a	0.57^b
	B (mg kg^{-1}TM)	4.36^b	4.49^b	4.77^b	4.73^b	4.66^b	4.69^b	4.85^b	5.58^a
	Ca/ B ratio	108.72	140.87	135.73	133.17	119.44	128.80	130.69	101.49

a. b. c - different letters indicate significant differences for the Index (%), Ca (g kg^{-1} TM) and B (mg kg^{-1} TM) ($p < 0.001$) depending on the cultivars

In 2005 the concentration of Ca and B is significant different between the varieties (Table 1). The Ca content of cv. Adretta is significant higher compared to cvs. Afra, Nicola, and Gala. In contrast the B concentration in cv. Gala was significant the highest. No relationship between Ca and B and blackspot index occurs. The blackspot susceptibility decrease after five month storage as well as the Ca- and B-concentration and increase again after eight month storage as well as Ca- and B- content but without relationship between the parameters. However, the Ca/ B ratio seems to be an indicator for blackspot susceptibility variations. In general, there are many suggestions about the role of this ratio in plant physiology. Plants with low Ca content are characterized by low B tolerance and Ca efficient plants demand high B concentrations (Bergmann 1993). In tubers of celery (*Apium graveolence)* Ca/ B ratios from 15 to 100 are normal and values from 100 to 300 indicate B deficiency (Bergmann 1993). At this level results of our research indicate after harvesting B deficiency in the cv. Adretta (Ca/ B ratio 107.4) (Table 1). The other cultivars do not suffer from B deficiency. After five months of storage a moderate Ca/ B ratio occurs (<100) in all varieties. After long time of storage of eight months blackspot susceptibility increase significant in all varieties except Marabel. At this time all varieties suffer from B deficiency (Ca/ B ratio > 100) but no relation between the Ca/ B ratio and blackspot susceptibility of cultivars was found.
With respect to the different varieties, an increase in the blackspot susceptibility was correlated with the specific gravity of the tubers (Pawelzik *et al.* 2005). The specific gravity is related to the dry matter and starch content of potato tubers (Iritani 1981, McNabnay *et al.*1999). Classes of specific gravity of tubers are influenced by climatic and cultural factors independent from the potato varieties (Davies, 1998). Tuber cells are more vulnerable to impact damage when they are stuffed with starch granules (Laerke 2001). The analysed tubers harvested 2005 had specific gravities from 1.055 to > 1.095 kg L^{-1}. The Ca content of freshly harvested tubers tends to decrease with higher specific gravity from 0.49 to 0.40 g kg^{-1} TM. However, a significant declining B concentration occurs from 5.8 to 4.73 mg kg^{-1} TM with higher specific gravity. With regard to the Ca/ B ratio no B deficiency occurs (<100).
A storage period of five month decrease the blackspot susceptibility of tubers but it is still significantly correlated with the specific gravity (r^2 =0,914) Moreover, the Ca content is significant negative correlated with specific gravity (r^2 =0,958). The B content also decrease but the relation is not significant. The Ca/ B ratio differs not significantly compared to fresh harvested potatoes. After storage of eight month the blackspot susceptibility is still significant correlated with the specific gravity (r^2 =0,941). Furthermore, Ca (r^2 =0,941) and B (r^2 =0,973) decrease in tubers with increasing specific gravity. Long term storage induces B deficiency in all evaluated tubers (Ca/ B ratio 120 – 130).
Summarising the literature Ca is known to prevent physiological disorders in potato tubers via stabilizing cell membranes (Laerke 2001). However, there is no significant relationship between Ca content and blackspot susceptibility. Boron might have the function to maintenance the Ca pectin association (Yamauchi *et al.* 1986) and therefore to enhance cell rigidity. The Ca/ B ratio depending on the mineral efficiency of the varieties and might effect the blackspot susceptibility of tubers.

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ESTIMATION OF POTATO PLANT PHYSIOLOGICAL STATE DURING GROWING PERIOD WITH IMPLEMENTATION THE TECHNIQUE OF CHLOROPHYLL A FLUORESCENCE

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Introduction

Potato as a plant of moderate climate is very popular and commonly cultivated in Poland. The prognosis of global climate warming enjoin us to special study making. The aim of our experiment was the estimation of potato plant physiological state during growing period with implementation the technique of chlorophyll *a* fluorescence.

Material and methods

The experiment was conducted in 2006 in Jadwisin, 34 km from Warsaw. Meteorological data were noted by automatic meteorological Campbell's station. Potato minitubers of different size of two potato cultivars: Tara (middle early) and Jasia (middle late) were planted on 23 April in the experimental field. From the beginning to the end of potato growth Plant Efficiency Analyzer HandyPEA (Hansatech Instruments Ltd., UK) was used to measure basic chlorophyll *a* fluorescence parameters: minimal fluorescence (Fo), maximal fluorescence (Fm), maximum quantum efficiency of photosystem II (Fv/Fm), variable fluorescence (Fv), time at which Fm occurs (Tfm), area over the curve between Fo and Fm (Area) and finally vitality index of PS II (PI). Water deficiency in the soil was alleviated by irrigation. The yield was determined in the middle of September after haulm destruction.

Results

The growing period characterized by small amounts of rainfall and very high temperature. The most unfavorable conditions for potato development were in July when average maximum temperature was 28,1^{o}C. Unfortunately during 12 days exceed 30^{o}C and reached 33,3^{o}C. Thermal conditions and soil moisture improved in August. In spite of the field irrigation in July a physiological state of plants fell into a decline, not depending on the cultivar and a size of seed tuber. The results of basic chlorophyll *a* fluorescence parameters which were measured during whole growing period showed that on 8th July took place a decrease of photosynthetic activity lasting during the time of high temperature stress. After improvement of thermal conditions the second growth was observed and mechanical haulm destruction was indispensable before harvest. Although the tuber yield was quite high (on the average 42,13 t·ha-^{1}), tuber physiological defects covered more than 50 % of the crop. The main were tubers physiologically young with not mature skin, as the effect of second tuberization.

Conclusion

The assessment of photosynthetic activity of potato plant during growing period with implementation the chlorophyll *a* fluorescence method may be helpful in estimation of plant physiological state under unfavourable climatic conditions and introducing of signalling about necessity of harvest acceleration for avoid the second growth and second tuberization.

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Quality and Processing

RELATIONSHIP BETWEEN CULTIVATION SYSTEM, POTATO TUBER FLAVOUR AND MACRO OR MICRONUTRIENTS CONTENT IN TUBERS

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Introduction

Organic farming is a sector of growing economic significance. The consumers of organic farming products have increasing demands for high quality and safety of these products. The question is whether the production applying ecological cultivation systems is able to offer products, which really meet consumer demands [2]. In the case of potato, the flavour of tubers and content of macro- and microelements seem to be factors, which can be readily assessed for tubers from various cultivation systems [1].

Materials and methods

The field experiments were performed under different cultivation regimes (i.e. high-input, traditional, low-input and organic system – Table 1). In each experiment, a set of table cultivars was planted. After harvest, content of macro- and microelements was evaluated, namely content of N (using titrimetric method), P (colorimetric method), K, Ca (flame photometry), Mg, Pb, Cd, Cu, Zn, Mn, Fe and Hg (atomic absorption spectrometry). Tubers flavour was assessed using EAPR procedure for encoded tuber samples from each experiment (=cultivation system).

Table 2. Description of cultivation systems

Cultivation system	Fertilizers			Plant protection			
	N	P	K	Herbicides	Fungicides	Insecticides	Dessicant
organic	manure			no	limited applying of copper fungicides	no	no
low-input	manure			no	copper fungicides	yes	no
traditional	93	91	170	limited applying	limited applying	yes	no
high-input	92	75	190	yes	yes	yes	yes

Results

The content of macro and microelements depended clearly on interaction of tested factors (i.e. cultivation system, cultivar and/or year) and on environmental factor (year). The influence of cultivation system was observed in the case of some elements. In tubers from low-input and organic systems lower level of nitrogen and higher level of phosphorus was observed as compared with traditional and high-input system (Table 2). The content of potentially harmful microelements was not related with cultivation system, but rather with location as it was observed in the case of cadmium and lead (Table 2). However, specific element content in soil was not related with its content measured in tubers.

Table 3. Mean macro and microelements content in tubers from plant grown under four different systems

Cultivation system	Mean for standardized values											
	N	P	K	Mg	Ca	Pb	Cd	Cu	Zn	Mn	Fe	Hg
organic	-0,12	1,17	0,03	0,62	0,11	0,47	-0,56	1,28	0,23	-0,26	0,53	-0,34
low-input	-0,41	0,28	0,68	-0,51	0,11	-0,57	-1,01	0,18	-0,65	-0,33	-0,52	-0,38
traditional	0,39	-0,64	-0,99	-0,03	-0,75	-0,33	0,64	-0,76	0,15	0,49	-0,49	0,77
high-input	0,14	-0,81	0,28	-0,08	0,53	0,43	0,94	-0,69	0,26	0,10	0,48	-0,06

Flavour of tubers depended on cultivar, cultivation system and on interaction of both factors. Flavour of tubers from plants growing under organic and low-input systems was better than for tubers from traditional and high input systems (Fig.1). This relationship was true only for some cultivars. The production of tasty tubers needs a careful selection of potato cultivars, which are suitable for specific cultivation system.

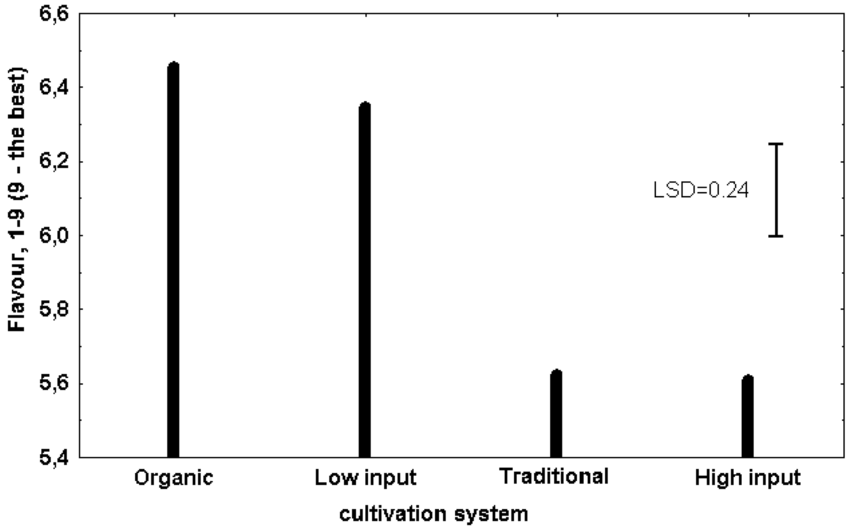

Fig. 1 Mean flavour of tubers from different cultivation systems

Good flavour of tubers was connected with low content of nitrogen, magnesium, zinc and manganese, but with high level of phosphorus (Table 3).

Table 4. Relationship between mean content of macro- and micronutrients and taste of tubers of tested cultivars

Flavour [1/]	Mean for standardized values											
	N	P	K	Mg	Ca	Pb	Cd	Cu	Zn	Mn	Fe	Hg
bad	0,42	-0,16	0,11	0,31	0,21	0,05	0,28	-0,11	0,48	0,50	0,23	0,21
poor	0,19	-0,05	-0,04	0,02	0,13	0,25	0,21	-0,05	0,25	0,01	0,21	0,15
good	-0,29	0,04	-0,08	-0,23	-0,31	-0,29	-0,27	-0,03	-0,41	-0,30	-0,42	-0,15
very good	-0,49	0,43	0,05	-0,10	0,25	0,43	-0,22	0,50	-0,36	-0,37	0,44	-0,33

[1/] Flavour of tubers was evaluated using1-9 scale (9 - very good taste). In this table, cultivars were grouped into four classes, according to the flavour of tubers, which was bad (flavour score <5,5), poor (5,5 – 5,9), good (6,0 – 7,5) or very good (>7,5).

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THE EFFECT OF A GLOBAL CLIMATE CHANGE ON THE PROCESSING QUALITY OF POTATOES

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Introduction

Potato processing has become a quite important part of the food industry. In some countries, the consumption of potato products has exceeded those of fresh potatoes.

The total number of products is substantial. Very often, an intensive heating step is included in manufacturing. Thereby, the Maillard reaction is involved, which describes a non-enzymatic reaction between free amino acids and reducing sugars (RS), e.g. glucose and fructose. Visible end products are dark coloured particles (melanoidins) and a number of volatiles and tasty compounds. Since 2002 it is also known, that the formation of acrylamide is linked to that reaction. On the other hand, the melanoidins shall have an antioxidant activity and anti-carcinogenic properties, too [Lindenmeier et al., 2002].

The concentration of RS in potatoes is the bottleneck of the Maillard reaction [Roe et al., 1990]. Therefore, potato processors control directly at the delivery point of the factory either the RS level or the intensity of the Maillard reaction with the option of a refusal. That is the reason why farmers themselves are interested to reduce the RS level as much as possible. Next to the choice of the best suited cultivar the specific growing conditions (e.g. fertilization and plant protection strategies) can be optimized. However, the weather is largely uncontrollable, only water deficiencies may be compensated by irrigation.

The scientific reports of a global climate change with enriched carbon dioxide concentration in the air, extremely temperatures and heavy rainfalls indicate some modifications of the biochemical pathways within cells. Among others, an intensified photosynthesis may result in higher yields but also in higher sugar levels [Stafford, 2007]. The latter case would lead to problems in potato processing.

Material and Methods

Taking this into consideration, a data set with seven cultivars of early and medium to late maturity classes, three locations (different soil types and weather conditions) and nine growing years was evaluated to find out some indication.

All potato samples were analyzed twice a year, just after harvest and after six months storage at +8°C and 95% rel. humidity. Next to a dry matter determination and an enzymatic sugar analysis (glucose, fructose and sucrose), all samples were crisped at a semi-technical processing line (peanut oil; +175°C until final moisture of 1-2%). Crisp colour was measured with a chromameter CR 310 (Konica-Minolta). Lightness (L*-value) was transformed to a characteristic note (1-10).

Results

A six months storage increased the reducing sugars about 67% and sucrose about 65% (average values). Sucrose was not correlated with crisp quality. Reducing sugars of early mature potatoes could predict crisp quality with 66%. Medium to late mature cultivars had a relative low coefficient of determination at harvest time (35%) and also after long term storage (40%). Differences between the growing locations had no significance within the statistical calculations. Single values are presented in Table 1. Dry matter content increased during storage.

The concentration of reducing sugars dropped down in the early maturing cultivars between 1998 and 2006 (from 140 to 112 mg RS/100 g fresh weight (FW)), whereas the concentration of sucrose slightly increased (from 347 to 364 mg sucrose/100 g FW). The sum of sugars investigated was largely constant (from 487 to 476 mg sugar/100 g FW). The same observation existed at the medium to late cultivars, whereby the effects clearly occurred after the storage period (from 141 to 54 mg RS/100 g FW; from 459 to 491 mg sucrose/100 g FW). Single years differed from the overall average with respect to individual atmospheric conditions (Table 2).

Tab. 1: ***Dry matter and sugar content of investigated cultivars as well as correlation toward crisp quality (RS: reducing sugars; FW: fresh weight).***

Object	Maturity category	Time of analyses	n	Average	Stddev.	Percentile		Correlation (r) with crisp quality
						5.	95.	
Dry matter content [%]	Early	Harvest	108	25.9	2.52	22.0	30.4	0.17
RS [mg/100g FW]			108	126	127	24.0	423	-0.81
Sucrose [mg/100g FW]			108	356	101	228	566	-0.25
Dry matter content [%]	Medium to late	Harvest	81	25.8	1.52	23.5	28.4	0.0
RS [mg/100g FW]			81	57.4	41.3	19.0	115	-0.59
Sucrose [mg/100g FW]			81	287	83.1	193	446	-0.09
Dry matter content [%]	Medium to late	Storage	80	27.1	1.67	24.6	29.7	-0.38
RS [mg/100g FW]			80	95.7	73.2	22.0	266	-0.63
Sucrose [mg/100g FW]			80	473	243	211	988	-0.32

Tab. 2: ***Significant variations of individual growing years to the overall average (level of significance: 95%).***

Object	Maturity category	Time of analyses	n	Conspicuous discrepancies
Dry matter content	Early	Harvest	108	2006 (reduced)
Reducing sugars			108	2000 (increased)
Sucrose			108	-
Dry matter content	Medium to late	Harvest	81	2006 (reduced)
Reducing sugars			81	2000 (increased), 1999 + 2003 + 2006 (reduced)
Sucrose			81	2005 (increased), 2001 (reduced)
Dry matter content	Medium to late	Storage	80	-
Reducing sugars			80	2000 (increased), 1998 + 2001 + 2003 + 2006 (reduced)
Sucrose			80	-

Discussion

Potato sugar analyses for several years gave evidence to an oligo-factorial dependency within processing cultivars. Next to the genotype growing location and growing year had an impact, too. Therefore, generalized conclusions are difficult. However, it can be concluded that the sucrose level may be high despite of a very low concentration of RS.

The investigated early mature cultivars had a partly high RS level. This indicates the necessity of a control for all incoming lots. With respect to the relative low correlation toward the final crisp colour, a frying test could be the solution (e.g. according to the Swiss baking test).

The observed trend of a RS-reduction is contrary to the forecast of some climatologists, who deduce from experiments with elevated CO2-concentrations enhanced potato yields, but also a dramatic change of the potato composition with enhanced low molecular weight compounds, sugars among them [Stafford, 2007].

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OSMOTIC PRE-TREATMENT EFFECTS UPON FAT UPTAKE AND SENSORY QUALITY OF FRENCH FRIES

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Osmotic treatment as a pre-step to further processing of plant and animal material allows to improve the nutritional and sensory quality of food products. Results of an experiment with French fries demonstrate the practicability of an osmotic pre-treatment. Sodium and potassium chloride were used in different concentrations and different dipping times between 1 and 10 minutes. The sensory quality pointed out a reduction of oil uptake and also a shortening of end frying. Moderate salt concentrations also had positive sensorical effects.

ULTRASOUND IMPROVES CHIP COLOUR OF POTATOES STORED AT 3℃

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Potatoes of processing (Norin 1) and fresh-consumption (May Queen and Irish Cobbler) were stored at 3℃ for three months. Potatoes were sliced to 1.5 mm thickness. Ultrasound was used for lowering reducing sugar content of slices. The aim of this study was to determine whether ultrasound leaching improves chip colour of 3℃-stored potatoes. Ultrasound for 30 min yielded light colour chips; ultrasound could improve much chip colour of the potatoes.

INTRODUCTION

The demands of the potato-processing industry often involve the long-term storage of potato tubers. Maintaining high-quality of processing potatoes over long periods is an important issue for growers and processors. Storage conditions must be set to minimize sprouting, respiration, dehydration and disease. The most important problem of the potato-processing industry is to maintain a desirable light-colored product. Dark- and uneven-colored chips and French fries are unattractive to the consumer and often have an undesirable flavor (Copp et al., 2000). It is generally agreed that dark color development in processed tubers is primarily due to the Maillard reaction between the aldehyde groups of reducing sugars and the free amino groups of amino acids during frying operations (Rodriguez-Saona et al., 1997).

Low-temperature storage helps to reduce problems of sprout growth and losses due to disease and rotting. However, the resultant low-temperature sweetening of the tubers reduces the chip quality within a short time period (Wismer et al., 1995).

Water blanching is used to reduce the content of reducing sugars. However, it needs high energy and water consumption. Thus, we attempted to use ultrasound instead of water blancing. However, no reports exist concerning ultrasound leaching of sugars of potato slices. Therefore, the present study was initiated to evaluate improvement of chip colour of 3℃-stored potatoes by ultrasound leaching.

MATERIALS AND METHODS

Source and tuber storage

Tubers of processing (Norin 1) and fresh-consumption (May Queen and Irish Cobbler) cultivars were harvested in September, 2006. After harvesting, these potatoes were stored at 3℃ and 90-95% R.H. for three months.

Sample preparation

Potatoes were washed and sliced to 1.5 mm thickness.

Ultrasound leaching

Leaching was performed with an ultrasound-cleaning bath (Kaijo Co. Ltd., Japan) with 600 W of potency and 38 kHz of frequency. The internal dimensions of the ultrasonic bath are 26 cm × 36 cm ×25 cm. The temperature in the bath was controlled at 20℃. Potato slices were put into the bath for a maximum of 30 min.

Sugar analysis

Sugar analysis of potato sliced was measured using HPLC (Hironaka et al., 1990).

Frying and colour measurement

Slices were fried at 180℃ for 1.5 min. The colour parameter (L* and a*) was measured with a Minolta Colorimeter (CR400).

RESULTS AND DISCUSSION

Figure 1 shows changes in reducing sugar content of processing (Norin 1) and fresh-consumption (May Queen and Irish Cobbler) potatoes at 3℃ storage. As shown in this figure, the reducing sugar content of potatoes increased much at 3℃ storage. Chip colours of the potatoes were presented in Fig. 2. Each cultivar showed dark colours after 3℃ storage of 90 days. Then, the L* values decreased rapidly in storage at 3℃ (Fig. 3). On the other hand, a* values increased in 3℃ storage (Fig. 4).

Figure 5 shows changes in reducing sugar content of potato slices during ultrasound leaching. Although each cultivar decreased much in reducing sugar content during leaching, leached slices had still reducing sugar content more than 0.25 % (Smith, 1956) which is an acceptable content for making white colour chips; ultrasound-leached potatoes had relatively high sugar contents. Leached potato slices showed light chip colours, especially 30 min-leached potatoes (Fig. 6). Ultrasound increased L* values (Fig. 7), and decreased a* scores (Fig. 8).

Concerning ultrasound extraction, Pen et al. (1959) found that ultrasound waves and the associated microturbance of cavitation bubbles near surface of solid could reduce the mass transfer boundary layer and therefore give rise to an efficient increase of the mass transfer. In the present study, ultrasound-leached potato slices treated by had relatively high reducing sugar contents (Fig. 5), but ultrasound gave light to potato slices (Fig. 6). Thus, sugars near a potato slice surface could be removed much by ultrasound.

This study revealed that ultrasound improved much chip colour of 3℃-stored potatoes. The present information is important and useful for potato processors because even fresh-consumption potatoes could be used for making chips.

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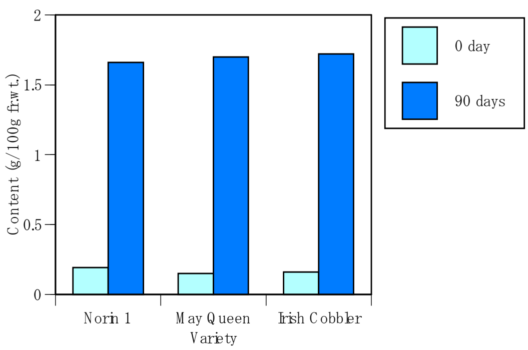

Fig.1 Reducing sugar content of potatoes stored at 3℃ for 0 and 90 days

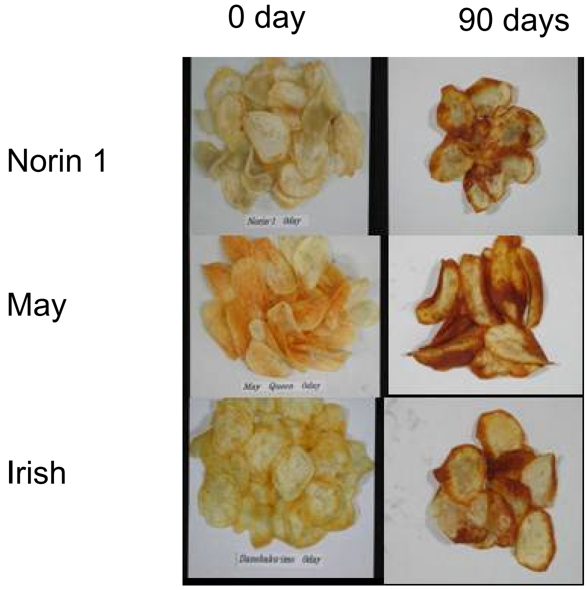

Fig.2 Chip colour of potatoes at 3℃ storage

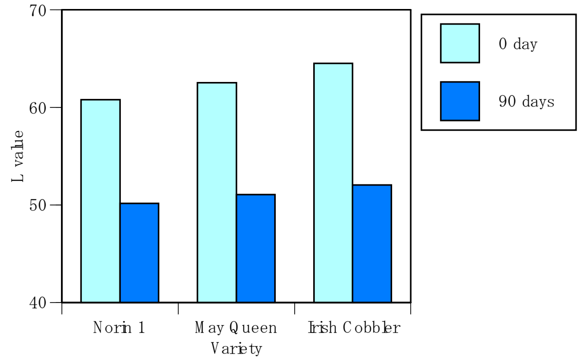

Fig.3 L value of potatoes at 3℃ storage*

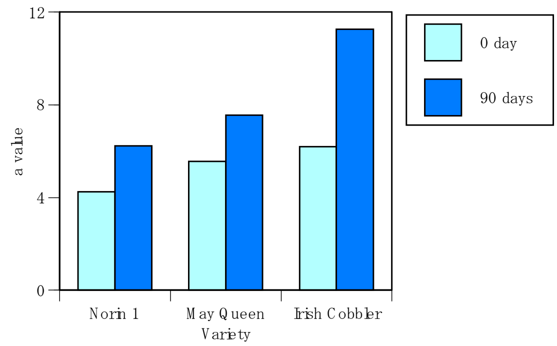

Fig.4 a value of potatoes at 3℃ storage*

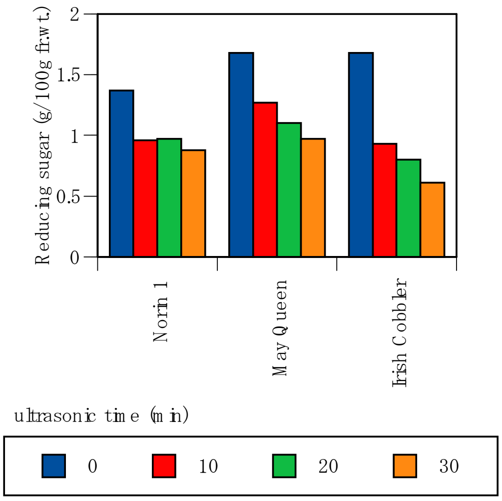

Fig.5 Reducing sugar content of potatoes dur ultrasound leaching

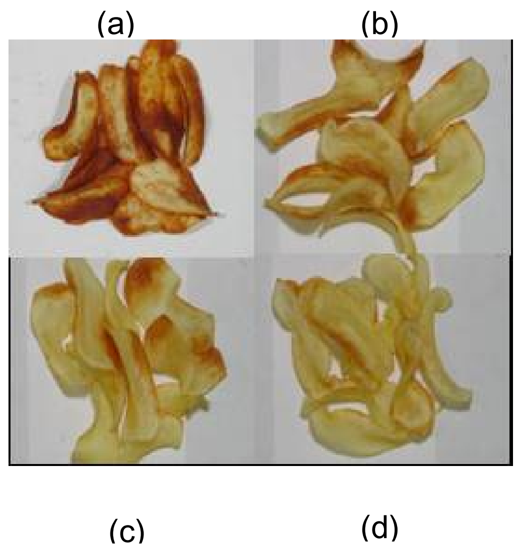

Fig.6 Change in chip colour during ultrasound leaching (a) ultrasonic time: 0 min (b) 10 min (c) 20 min (d) 30min

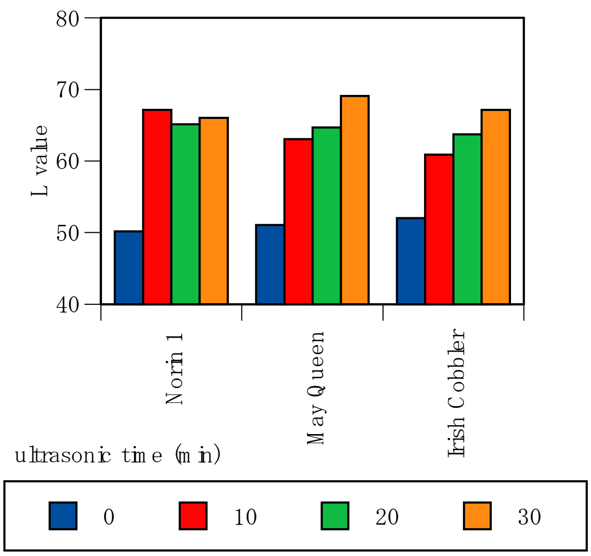

Fig.7 L value of potatoes during ultrasound leaching*

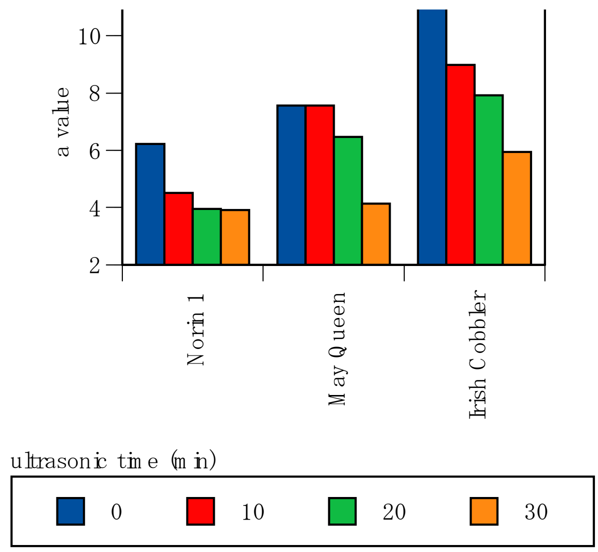

Fig.8 a value of potatoes during ultrasound leaching*

Storage and mechanization

EFFECTS OF DIFFERENT ETHEREAL OILS ON SPROUT INHIBITION AND TUBER SETTING OF POTATOES

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Keywords: Ethereal oil, sprout inhibition, storage losses, tuber setting

Summary

In the investigation period 2005/06 and 2006/07 the storage losses of five varieties were decreased both by caraway and peppermint oil (ethereal oils) as well as by the chemical sprout suppression (CIPC) compared with the untreated control. The oil of peppermint applied in the ULV technique at store filling had no sufficient effect on sprout inhibition for the whole storage period. However, the weekly application of caraway oil (carvon) using a cold fogging applicator showed a sprout inhibition comparable with CIPC and decreased silver scurf infections. In field trials the storage samples treated with carvon had positive effects on the growth development. There were different effects of the ethereal oils on the tuber setting depending on the variety, but the yield was not influenced. The treatment just before storage with peppermint oil showed moderate, an additional application after storage time may cause dwarfing effects. The application of carvon is connected with high middle costs and assumes a storage with forced ventilation system.

Introduction

In the potato production ethereal oils could be used before or during storage time for sprout inhibition. Against chemical active substance Chlorpropham (CIPC) ethereal oils show a temporary, i.e. reversible sprout inhibition, so a treatment of seed tubers might be possible. These oils might have a fungicide effect against storage diseases, like silver scurf, as well as positive effects on growth development and tuber setting in the field. The oil of peppermint is applied in the ULV technique at store filling, the application of the carvon occurs while using a cold fogging applicator weekly during storage time and assumes a forced ventilation system. For an economic use of ethereal oils the effects on storage losses and growth of the potatoes in the field must be known.

Materials and methods

In two storage periods from 2005 to 2007 potatoes of five varieties with different dormancy and tuber setting were stored in letter boxes with forced ventilation system. Beside an untreated control the oil of peppermint as well as the chemical control with Chlorpropham (CIPC) were applied in the ULV technique just before storage. The weekly application of the carvon using a cold fogging applicator occurred from end of November (2005) and end of October (2006). The dosage of the carvon was applied according to manufacturer's data. The potatoes were stored over 6 months with a storage temperature of 5 °C. After storage time in the middle of March weight losses, sprouting, dry and soft rot as well as fungal diseases like silver scurf infections were ascertained. Before planting a part of the samples treated with peppermint oil in autumn received an additional application. In a field trial the untreated control as well as the treatments with the peppermint oil (two treatments: autumn and autumn/spring) and carvon were tested for emergence, growth development, tuber setting, yield and quality.

Results and discussion

On average of 5 varieties the storage losses were reduced by the biological and chemical sprout inhibition in comparison to untreated control (Fig. 1). Nevertheless, the single varieties reacted very differently on the different treatments. The carvon achieved a sprout suppression comparable with

Chlorpropham for the whole storage period of about 6 months. Against it the application of the peppermint oil showed no sufficient sprout inhibition in the whole storage period. The effects of the ethereal oils on weight losses were influenced by the varieties. Silver scurf infections decreased especially by the carvon in comparison to untreated control.

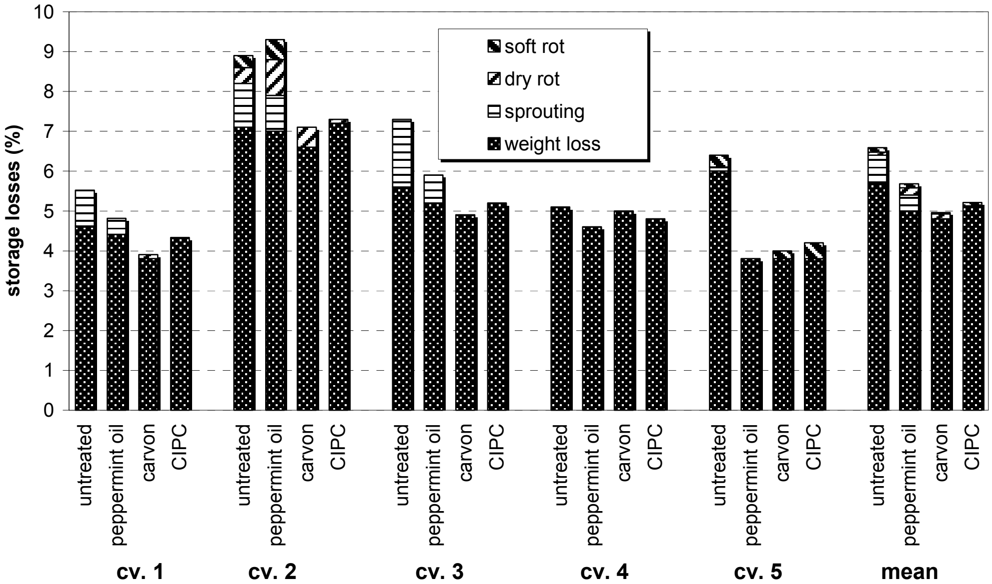

Fig. 1: Storage losses of 5 varieties (2006/07)

In a field trial after storage time the ethereal oils showed different effects on growth development with clear differences between the varieties: the peppermint oil had moderate effects on plant growth, an additional application just before planting might cause dwarfing effects and the carvon improved plant growth development. Depending on variety there were different influences of ethereal oils on tuber setting (Fig. 2). Nevertheless, yield and grading were not influenced by the ethereal oils.

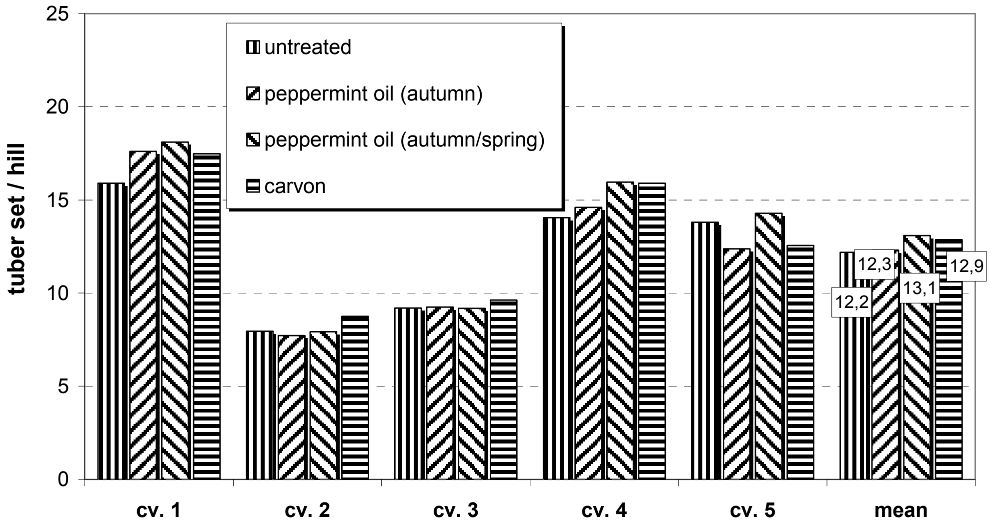

Fig. 2: Tuber set of 5 varieties (2007)

For an economical use of carvon with costs about 40 €/t benefits on storage losses and tuber setting of the different varieties must be examined. Besides, the smell of carvon after storage gets weaker very slowly.

EFFECT OF MALEIC HYDRAZIDE AGAINST REGROWTH OF TUBERS AFTER A STRESS PERIOD

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Abstract:

Second growth is a physiological disorder of potato which occurs more or less often in relation with the variety sensitivity after the crop was stressed during the vegetation phase by hot temperatures, especially during a dry period. A survey carried on Bintje cultivar during the hot summer 2006 by a technical working group (Mc Cain, Agricultural Chamber and ARVALIS-Institut du végétal) on 13 fields partially treated with Maleic Hydrazide (MH) 3200 to 4000 g a.i./ha showed globally a beneficial effect of MH to reduce the impact of this physiological problem. A significant reduction of second generation's tubers was noticed. The major favourable impact of the product was observed in the reduction of the floating tubers of low gravity in salted bath, an important quality parameter controlled at the entrance of French fries factories. This induced a significantly higher commercial yield for treated parts of the fields. Other technological quality parameters (dry matter, after frying colouration) were also optimised. In the survey's conditions, an economical approach indicated the profitability of the treatment for the large majority of the fields.

Key words
Floating tubers, Maleic Hydrazide, physiological disorder, regrowth, salted bath, second generation

Introduction

Regrowth is a physiological disorder which appears regularly in warm seasons with Bintje cultivar, very sensitive for this phenomenon when it is grown without irrigation supply. This was the case in 2006 with particularly hot weather in July in Northern France. The presence of second generation tubers in the harvest induces more or less important problems for storage and further on for commercialization, especially for a processing utilization: too low dry matter content for some tubers and glassy tubers containing high level of reducing sugars. In order to reduce the bad effect of these factors, salted baths are used by the French fries factories in order to eliminate the tubers with too low density.

At the end of July 2006, observing the risk and the start of the apparition of a second growth problem on Bintje fields cultivated for a French fries destination, a survey project was decided by Mc Cain, Nord Pas de Calais Agricultural Chamber and ARVALIS- Institut du végétal in order to examine the possible interest of Maleic Hydrazide (MH), a growth regulator registered under the commercial name Fazor by the Company Chemtura. It is applied on the foliage during the vegetation period in order to avoid sprouting during the first months of storage by affecting the cells multiplication.

Material and Methods

In agreement with the concerned growers, 13 commercial fields of Bintje variety were chosen in the Northern part of France for receiving an application of MH (3200 to 4000 g a.i/ha) done on the crop at the end of July / beginning of August after the coming back of rains following a 3 weeks period of dry and hot weather (maximum temperatures up to 35 °C). At these dates regrowth was often appearing with the presence on some tubers of "sprouts" or even second generation tubers of small size. In each field an area was kept free of any treatment in order to have an Untreated Reference in the same condition of production.

After the application, the rains were quite numerous and important over the whole region covered by the survey (globally minimum 120 mm in 3 weeks).
The foliage of these commercial fields was protected against late blight till mid to end of September, time when growers proceeded to foliage desiccation.
After a two to three weeks period of remaining the tubers in soil, a sampling of MH treated and Untreated zones was done by collecting 3 plots of 30 plants per condition.

The collected tubers were dispatched at the harvest on 6 different classes in relation with their physiological appearance regarding regrowth comportment:

- Class 0 : no symptom of regrowth ("normal" first generation tuber)
- Class 1 : tubers of first generation with a well developed second generation tuber
- Class 2 : tubers of second generation
- Class 3 : tubers of first generation with "sprouts"
- Class 4 : diabolos (2 tubers joint with a large connection difficult to break)
- Class 5: "dolly" tubers (a big first generation tuber supporting closely one or two small tubers less than 20 mm with a short connection of a few millimetres).

Each category was counted and weighted. For sufficient weights of 50+ tubers, dry matter content was evaluated by density under water measurement. The number and weight of tubers of low density were also noted after a separation from others through a salted bath (d= 1,060) in order to evaluate also the commercial part of the yield, able to be used for Mc Cain French fry factory. Colouration of fried products was also noted using USDA table.

Results and discussion

1. **Physiological effect**

The physiological disorders concerned from 10 to 80 percent of tubers from the Untreated part of the all fields. Even variable, this comportment shows that the impact of regrowth was real on Bintje crop in 2006 in non irrigated fields from northern France (figure 1).

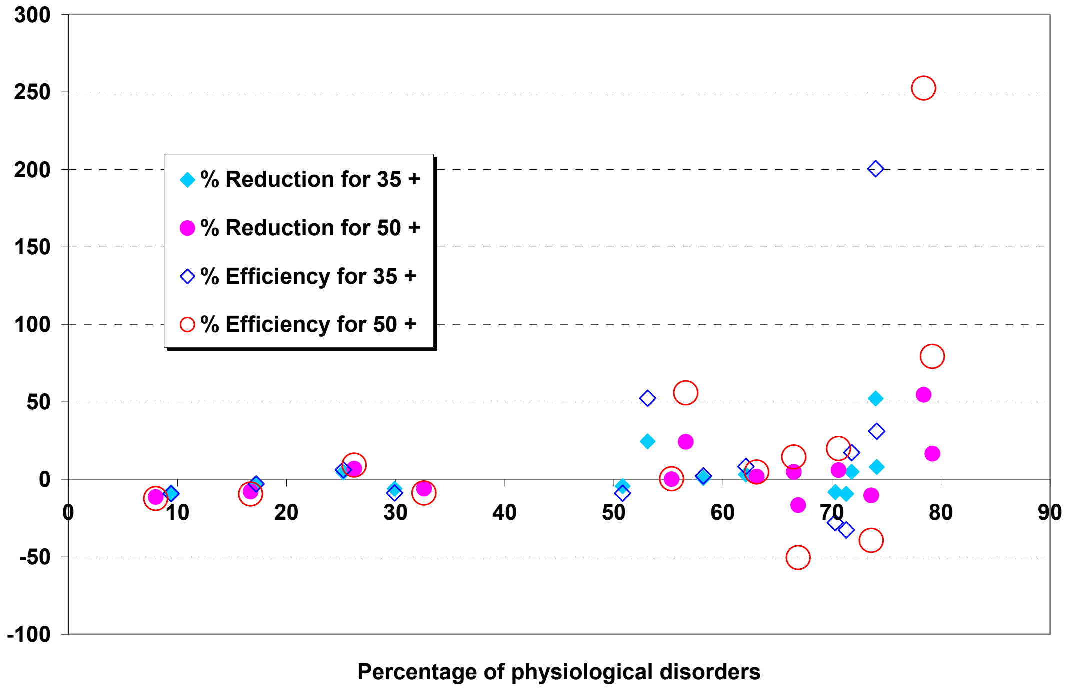

Figure 1: Effect of MH application on the reduction of physiological disorder and MH efficiency on this parameter in relation with the importance of physiological observed on tubers

In these conditions the application of MH gave favourable effect to reduce the physiological disorders for 54 % cases regarding tubers larger than 35 mm (35+) and 62 % cases for 50+. Globally the decrease of the different apparent disorders was low: -4,5 % (35+) and 4,8 % (50+) with MH use but it reduced significantly the number of 2nd generation tubers of 7,2% (35+) and 8,5% (50+).

It seems that the bad efficacy of MH observed on a few fields was in relation with very hard environmental conditions for the plants at the time of application in relation by example with extremely bad soil structure (compaction under tillage) or deficient nitrogen balance. These induced too high level of stress for the plants which couldn't have good product absorption.

2. Raw yield results

For 62 % cases, the raw yield of treated plots was increased with and average of +0,5 t/ha (35+) and +2,1 t/ha (50+). The cases of raw yield reduction were more often observed in the situation of severe second growth symptoms (more than 60 % tubers affected).

3. Floating tubers

Salted baths (d=1,060) are regularly used to separate and to eliminate the tubers with too low gravity at their entrance in the factory, especially when the risk of physiological disorders is heavy in the fields. On this point, the beneficial effect of MH was observed in most cases (77 to 85 %) with a main significant reduction of 5,2 % floating tubers for 35+ and 4,6 % for 50+ (figure 2).This positive action of MH was observed as for quite low as for quite high severities of regrowth.
Complementary controls of residues concentration in tubers done for a few discriminative fields showed a good positive relation between the residues level and the efficiency of MH in the reduction of floating tubers.

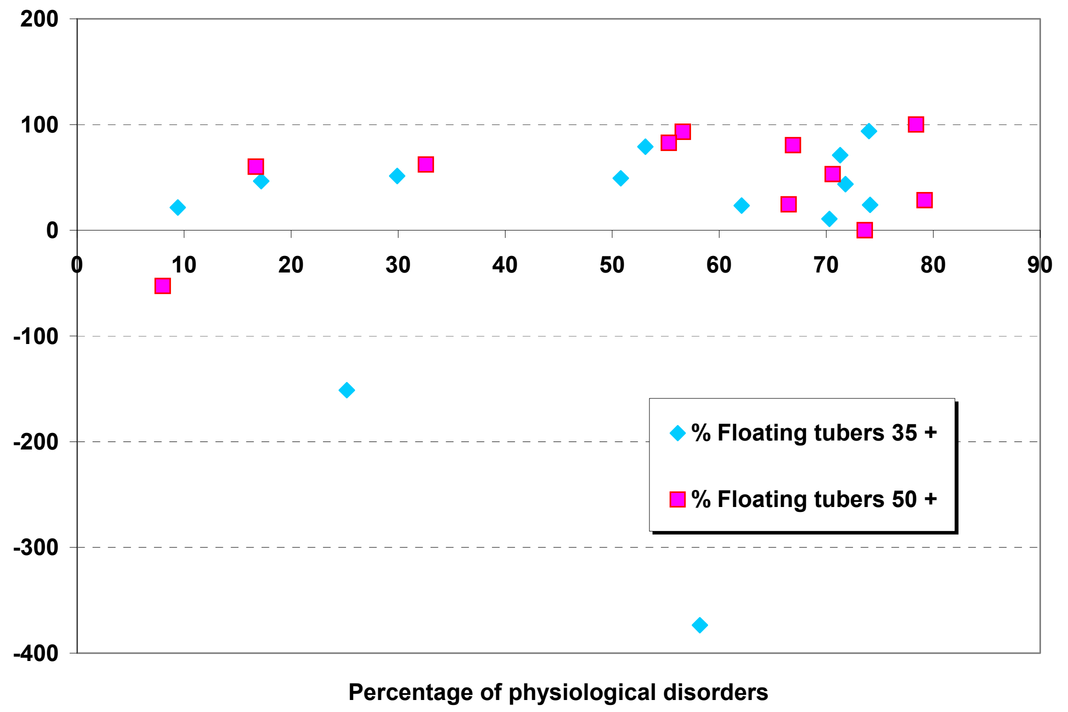

Figure 2: Efficiency of MH application on the reduction of floating tubers in comparison with the Untreated condition in relation with the importance of physiological disorders observed on tubers

4. Commercial yield

The combination of the two previous factors gave positive results for the commercial yield (raw yield minus floating tubers) of 69 % (35+) to 77 % (50+) fields' parts which have received an MH treatment, corresponding in MH efficiency till 44 % on this parameter. Dispatched on the 13 fields, the positive effect of MH treatment gave a significant increase for the commercial yield of +3,1 t/ha (35+) to +3,5 t/ha (50+) (figure 3).

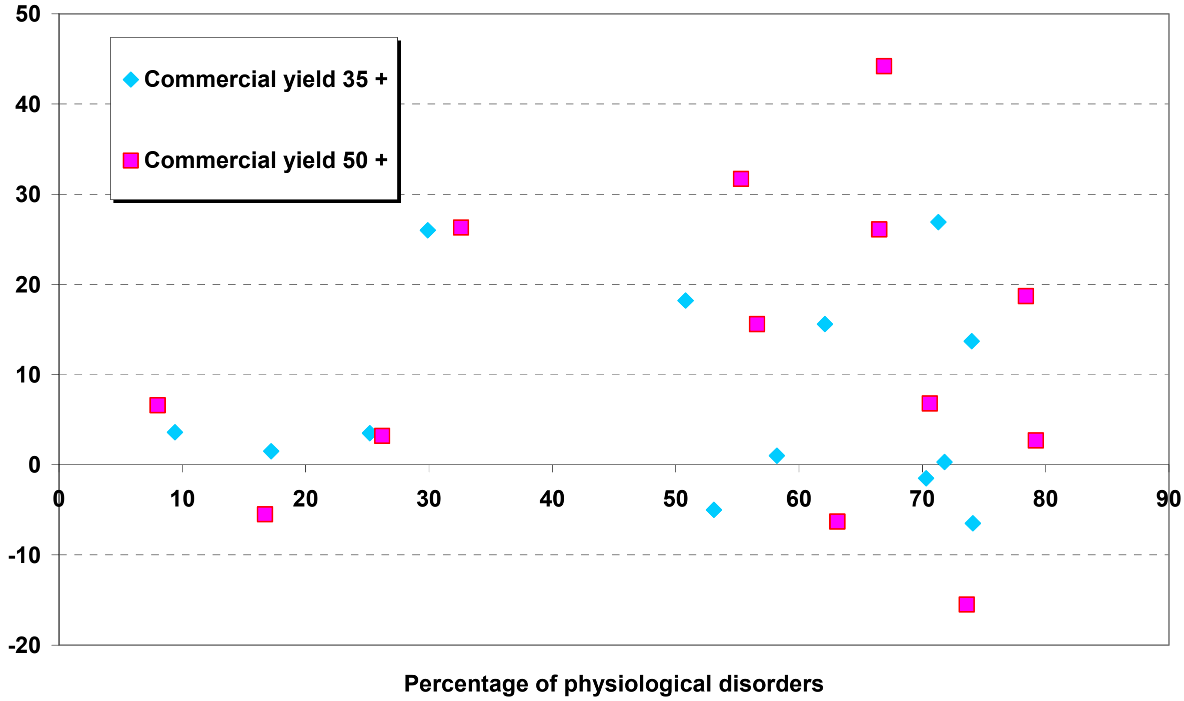

Figure 3: Efficiency of MH application on the commercial yield in relation with the importance of physiological disorders observed on tubers

5. Quality data

As for dry matter content than for frying index, together measured on 50+ tubers, the MH treatment gave a main positive but non significant effect : an increase of + 0,5 % for dry matter content and a reduction of 0,4 point for the colouration index (USDA).

6. Economical aspects

In a first examination, the cost for the application, around 135 €/ha (chemical +spraying), was covered in 70 % survey's fields by the commercial yield observed after the elimination of floating tubers.

In a second way, regarding the aspect that the processing factories only accept at the entrance a maximum level of floating tubers (10 to 16%) which can be eliminated by the plant, the non application of MH could require a preliminary salt bath on the farm completely paid by the grower for 4 to 5 cases over the 13 fields of the survey (estimated cost 525 €). With MH application, this pre-treatment would be only demanded for 1 to 2 cases, corresponding so at a 60 to 70 % reduction.

Conclusion

In the specific conditions of the survey carried on Bintje in the summer 2006, the application of Maleic Hydrazide just after the apparition of the risk of regrowth gave mainly favourable results, especially for the reduction of floating tubers in salted bath which are not acceptable for the processing destination and often at the origin of storage problems. In these cases the cost of the application was covered by the increase of the commercial yield.

The observations done when bad MH efficacy was noted show the necessity to have plants in a not too bad vegetative condition and sufficiently good soil structure in order to maximise the well distribution of the product to the tubers and the better efficacy of MH against regrowth.

INVESTIGATION ON THE WORK OF A FINGER REFLECTION-FRICTIONAL SEPARATOR FOR POTATOES

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The work indices of a Finger Reflection-Frictional Separator Stand were investigated by artificially created samples, consisting of 50 potato tubers of cv. Sante, 50 similar-size spherical stones and 50 dry clods. In one pass sample processing, the stand achieved an integrated separation coefficient of up to 95,3 %, without creating a risk of potato damage. The calculated output showed that the separator had a capacity of one pass processing of the whole second stage separation mass at potato harvesters or for substitution of 2 ÷ 3 body inspection units with 8 ÷ 11 farm-hands. The results obtained should be confirmed under real production conditions.

INTRODUCTION

Many units for separating potato tubers from stones and clods of a similar size and shape have been developed, but most of them usually detach satisfactorily one type of impurities only. The electronic separators detach all the impurities, but the necessity of body signalization and identification limit their output, moreover their disturbance susceptibility leads to separation errors. For that reason, the research for the development of an effective separation unit continues world-wide [4].

Both mechano-mathematical and simulation models of the Finger Reflection-Frictional Separator (FRFS[2]), as well as the investigation on the unloading mode of its delivery conveyor confirm its separation capability in case of single body delivery [1, 2, 3].

OBJECTIVE, METHOD AND MATERIALS

The main aim of the investigation was the establishment of the FRFS work indices during flow delivery of a mixture, which consisted of potato tubers and stones and clods of a similar size and shape.

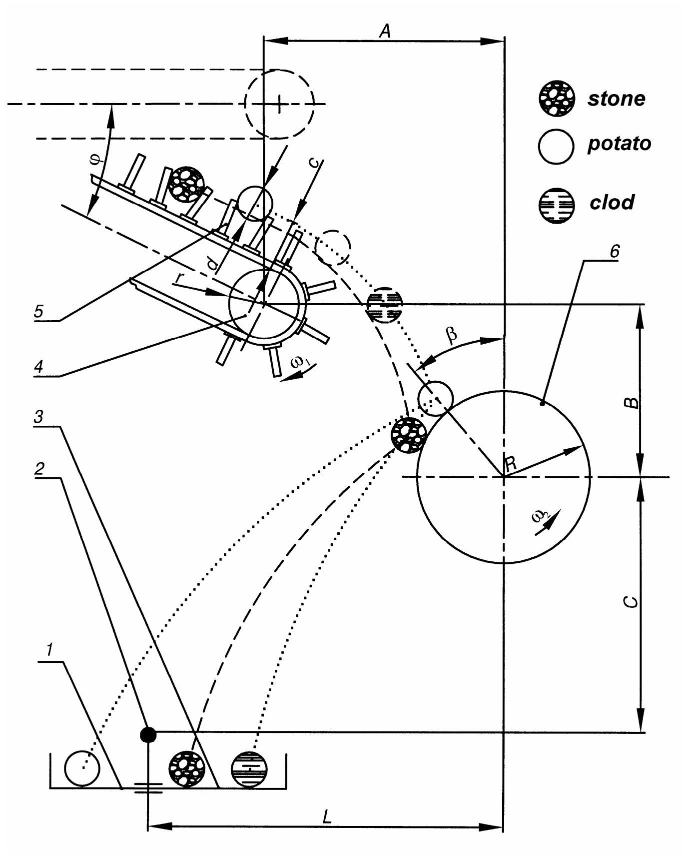

Figure 1. Scheme of the Finger Reflection-Frictional Separator experimental stand
1- **container for potatoes,** ***2*** **– divider,** ***3*** **– container for impurities,** ***4*** **– feeding conveyor drum,** ***5*** **– Pace's finger belt,** ***6*** **– reflection-frictional drum.**

The object of the investigation was the FRFS experimental stand (Fig. 1), which was an upgrade unit of the Reflection-Frictional Separator [1, 3]. The FRFS operation was based on four separation parameters. The first was the difference between the volume densities of both potato tubers and similar-size spherical stones. This parameter led to their different sinking depths ***c*** among rubber fingers of the Pace's belt *5* [5] and to the deviation of their trajectories before the impact on the drum *6* and after that, due to alteration of their contact angles ***β***. The difference between take off modes from delivery

[2] **FRFS - Finger Reflection – Frictional Separator**

conveyor of both tubers and stones was used as a second separation parameter for increasing the trajectory deviation. The chosen modes ensured centrifugal forces domination into the potato and gravity forces domination into similar size stones [3]. The third parameter was the difference between rebound coefficients of tubers and clods and the fourth - the difference between their instantaneous friction coefficients. The last two parameters led to the clod trajectory deviation from the tuber trajectory after their contact with the drum as in the Reflection-Frictional Separator [1].

The investigation was conducted through B_3 design experiment in three replications with artificially created samples, each of them consisting of 50 Sante cv. potato tubers, 50 similar-size spherical stones and 50 dry clods. The controlled factors (Table 1) were the delivery conveyor rotational frequency - n_1; the average diameter of each body in the sample - d and the longitudinal angle of the delivery conveyor - φ. The specific mass feeding Q was presented as an integral factor, because it showed the number of feeding bodies per second on a meter working width. It was calculated by the formula:

Table 1. Variation levels of controlled factors.

Levels	Factors		
	n_1, [min^{-1}]	d, [mm]	φ, [°]
Up	**100**	**70**	**0**
Medium	**70**	**50**	**7**
Down	**40**	**30**	**14**

(1) $$Q = \frac{N}{t.b_w}, \ [\textit{number of bodies /s.m}],$$

where:

N – the number of bodies in each sample;

t – the sample processing time, [s];

b_w – the separator working width, [m];

The responses were the number of the wrongly separated tubers, stones and clods in the containers *1* and *3*, which were used to calculate the following indices:

(2) $$\varepsilon = 100 - (\delta_p + \frac{\delta_{ss} + \delta_c}{\lambda}), \ \text{at} \ \lambda \geq 1 \ \text{or}$$

(3) $$\varepsilon = 100 - (\lambda.\delta_p + \delta_{ss} + \delta_c), \ \text{at} \ \lambda \leq 1;$$

(4) $$W_h^T = 3,6.Q.b_w.m \ ,$$

where:

ε - the general separation coefficient, [%];

λ - the impurity – tubers ratio in the experimental samples;

δ_p - the partition of the wrongly separated tubers, [%];

δ_{ss} - the partition of the wrongly separated spherical stones, [%];

δ_c - the partition of the wrongly separated clods, [%];

W_h^T - the theoretical output per hour of the separator, [t/h];

m - the mass of the bodies in each sample, [kg].

In the present investigation, the tubers in the container *1* were submitted to shocks by the falling bodies. This event does not usually exist in potato mechanization and would not be accurate to determine potato damage index by the conventional method. Therefore in that case, the correlation between the potato damage risk and the tuber shock velocity was used to evaluate the injury risk for the separated production. The tuber shock velocities were determined by a computer simulation model [2].

The other FRFS parameters preserved the same values as the Reflection-Frictional Separator [1]. A regression analysis at 0,05 level of significance was applied for the experimental data. The FRFS quality indices were compared to the agro-technical requirements for potato harvesters [4]. The output was calculated for such a working mode at which the separator reached the quality requirements and a comparison between the main working indices of the FRFS and of the existing separators was made.

RESULTS AND DISCUSSION

The investigation was conducted with samples described in Table 2 and at impurity – tubers ratio of $\lambda=2$. The quality indices of the FRFS varied in the following intervals: $\delta_p=2,1\div5,3$ %; $\delta_{ss}=3,5\div75,9$ % ; $\delta_c=1,8\div8,6$ % ; $\varepsilon=52,1\div95,3$ % , which had the corresponding deviations *1,2÷4,2 %; 1,4÷16,3 %; 0,3÷3,7 %; 2,9÷13,6 %* . The following regression equations were obtained:

(5) $$\delta_p = 3{,}3850 - 0{,}5514.\varphi + 0{,}0349.\varphi^2 + 0{,}0003.n_1.d\ ,\ R^2=0{,}942,\ p(F)=6{,}5.10^{-7};$$

(6) $$\delta_{ss} = 0{,}5791.n_1 + 0{,}2097.d - 0{,}0389.n_1.\varphi\ ,\ R^2=0{,}911,\ p(F)=4{,}42.10^{-6};$$

(7) $$\delta_c = 4{,}2544 + 0{,}7697.\varphi - 0{,}0685.\varphi^2 + 0{,}00031.n_1.d\ ,\ R^2=0{,}858,\ p(F)=5{,}9.10^{-3};$$

(8) $$\varepsilon = 94{,}2715 - 0{,}2244.Q - 0{,}00203.d.Q + 0{,}0216.\varphi.Q\ ,\ R^2=0{,}95,\ p(F)=2.10^{-5}\ .$$

Table 2. Separation Mass Parameters

Experimental bodies	Average diameter, d, [mm]	Shape Coefficient k_f	Velocity Recovery Coefficient, k_v	Instantaneous Friction Coefficient, $k\tau$	Volume Density ρ, [kg/m^3]
Potato tubers, "Sante" cv.	**40 ÷ 69,5**	**1,3 ÷ 1,6**	**0,50**	**0,86**	**1062 ÷ 1087**
Spherical Stones	**40 ÷ 70,5**	**1,2 ÷ 1,5**	**0,52**	**0,89**	**2479 ÷ 2635**
Soil Clods, 8 – 9 %Humidity	**40,3 ÷ 71,2**	**1,3 ÷ 1,6**	**0,20**	**0,72**	**1340 ÷ 1515**

The minimum values of the coefficients δ_p, δ_{ss} and δ_c as well as the maximum of the general separation coefficient ε were obtained at specific feeding ***Q=40 bodies/s.m,*** conveyor longitudinal angle φ**=14°** and an average body diameter ***d=30 mm*** (Fig. 2, Fig. 3). Processing of larger bodies led to the decrease of the general coefficient mainly due to the increase of the wrongly separated stones, but it remained close to the requirements.

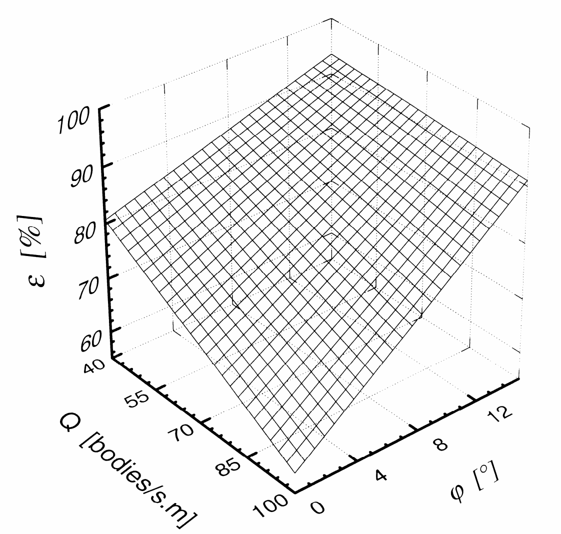

Fig. 2. Graphic of the integral separation coefficient ε depending on the specific feeding Q and the conveyor longitudinal angle φ at an average body diameter *d=70 mm* and an impurity – tubers ratio λ=2.

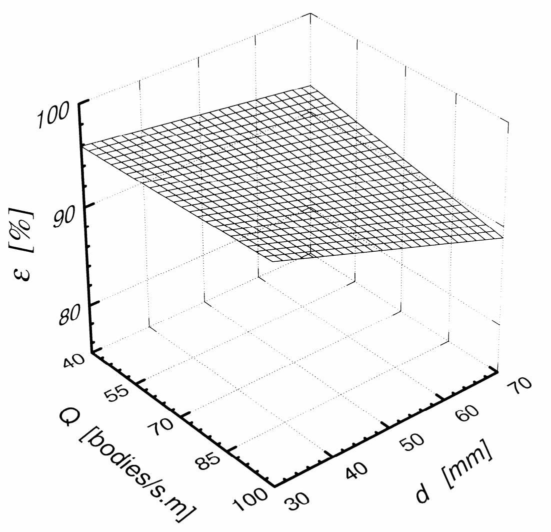

Fig. 3. Graphic of the integral separation coefficient ε depending on the specific feeding Q and the average body diameter d at a conveyor longitudinal angle φ=14° and an impurity – tubers ratio λ=2.

It should be noted that under real conditions the clod humidity is higher and the separation mass is not divided into three fractions like in the experiment. Therefore, changes of the integral coefficient in both directions could be expected. Contrary to the Reflection-Frictional Separator, the FRFS met quality requirements not only for clods, but also for stone separation, however, at 2,5 times lower specific feeding. If the FRFS feeding was increased up to ***Q=100 bodies/s.m*** like in its predecessor the general coefficient decreased to ***88 %*** (Fig. 3). This was due to the change from gravity to centrifugal mode of the body take-off from the conveyor when increasing its rotational frequency n_1 [3].

The potato damage probability was judged by the speed of the larger bodies because they were under the biggest load pressure and were the most susceptible to mechanical damage [5]. The three bodies moved at different trajectories (Fig. 4) and speed (Fig. 5) after taking off. The trajectories intercepted each other, that could cause possible hits among the bodies leading to separation errors, but it was less possible because of the bigger distance between the bodies due to the velocity increase. The specific feeding Q should be restricted for avoiding the body hits near the reflection drum. In the present experiment the tuber hit against the drum with a velocity of ***2,61 m/s*** and against the container *1* with ***3,62 m/s***. The first hit did not suppose any potato injury but the second imposed the usage of shock absorbing units [4]. The damage risk became maximal when the wrongly separated bodies dropped in container *1* where the stone had a speed of ***3,73 m/s*** and the clod ***3,47 m/s*** but in practice those hits could be avoided by choosing an appropriate speed of the taking conveyors [1].

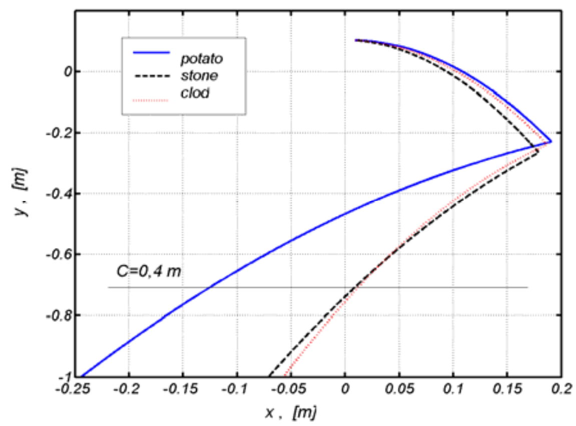

Fig. 4. Drawings of simulation results for the motions of the potato tuber, the spherical stone and the clod with an average diameter of d=*70 mm* at FRFR.

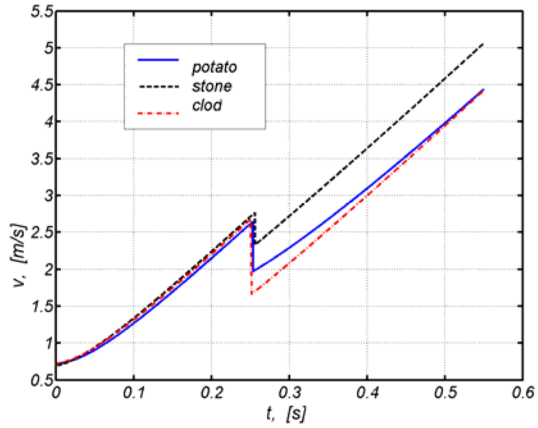

Fig. 5. Drawings of simulation results for the velocity of the potato tuber, the spherical stone and the clod with an average diameter of *d=70 mm*.

The FRFS reached the quality requirements at specific feeding ***Q=40 bodies/s.m*** and at a ratio ***λ=2***, while one-row potato harvester had to separate from 5 to 38 bodies per second. Obviously, the FRFS could process this mass without widening of the potato harvester. This conclusion is valid for the two- and more row potato harvesters as well. On the other hand, a single worker could separate ***1,2 ÷ 1,5*** bodies per second in average but either potatoes or impurities [4]. Consequently, the FRFS could substitute 2 ÷ 3 body inspection units with 8 ÷ 11 farm-hands. In case tubers, stones and clods have an average mass of ***0,073 kg, 0,135 kg*** and ***0,105 kg***, respectively, the FRFS could reach calculated output of W_h^T ***=15 t/h*** and it could meet the requirements for the separation quality.

The work indices of the FRFS were compared with those of both the mechanical separator "KS - 150ZJ" made by the Dutch company Bjilsma-Hercules and the electronic separator "Samro - 8024" of the Swiss company Bistronik [4]. The data showed that the simple FRFS reached the quality indices not worse than the mentioned separators. Obviously, the FRFS could be a base for the development of an effective separating machine. More over, there are additional opportunities for the increase of the quality indices by improving the sinking of the stones among the fingers of the feeding conveyor.

CONCLUSIONS

When processing a mixture consisting of equal number of potato tubers, stones and clods of similar shape and size at specific feeding of 40 bodies per second and one meter working width, the Finger Reflection-Frictional Separator stand reached ***2,1 %***, ***1,8 %*** and ***3,5 %*** wrongly separated tubers, clods and stones, respectively, and, a general separation coefficient of ***95,3 %***. Its calculated output was enough for one pass processing of the whole second stage separation mass at potato harvesters or for substitution of 2 ÷ 3 body inspection units with 8 ÷ 11 farm-hands. The simple constructed FRFS stand can be a base for the development of an effective separating machine.

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Posters

AGRONOMY

RADIATION USE EFFICIENCY AND ITS RELATION TO WEED COMPETITION IN POTATO

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Abstract

To study the competitive effects of redroot pigweed (*Amaranthus retroflexus*) and lambsquarter (*Chenopodium album*) on potato, an additive experiment was conducted in the spring of 2004 and 2005 in split-split plot based on randomized complete block design with 4 replications at the Seed Potato Production Station of RAN in Firouzkooh**.** Treatments were included 2 weed species in main plots (*Amaranthus retroflexus* and *Chenopodium album*), weed density in sub plots (2, 4 and 8 plants per meter of row) and relative time of weed emergence in sub-sub plots (8 and 4 days prior to potato and the same time with potato emergence in 2004 and the same time with potato, 2 and 4 weeks after potato in 2005). Results showed that by inceasing weeds interference, light interception of potato was reduced. So, in 8 plant of lambsquarter per meter of row , potato light interception was reduced 43.3 and 53.5 percent in 2004 and 2005. In third level of redroot pigweed (8 plants per meter of row) reduction of potato light interception was 56.5 and 66.8 percent in 2004 and 2005, respectively. By 2 redroot pigweed per meter of row, RUE of potato increased 2.8 percent in comparison with control. It means redroot pigweed supressd potato by shading. In the first and second levels of density of lambsquarter (2 and 4 plants per meter of row) results were similar. Average of potato RUE were 1.56 and 1.83 g/MJ intercepted PAR (for redroot pigweed treatment) and 1.72 and 1.89 g/MJ intercepted PAR in 2004 and 2005.

Keywords: potato, redroot pigweed, lambsquarter, competition, RUE.

Materials and Methods

Field experiments were conducted at the research station of seed Potato Production of RAN in Firouzkooh (33º 55' N, 52º 50' E and 1975 m mean sea level) in 2004 and 2005. The soil of the experiment plots was silt loamy in texture, pH=7.6; EC=1.23 mmose/cm; organic carbon percentage=0.5%; absorbable phosphorus = 20 ppm; absorbable potassium = 349 ppm and total Nitrogen = 0.05%. This experiment was done in split-split plot based on randomized complete block design with 4 replications. Individual plots size was 3 m wide by 16 m long. Treatments were 2 weed species in main plots (*A. retroflexus* and *C. album*), weed density in sub plots (2, 4 and 8 plant per meter of row) and relative time of weed emergence in sub-sub plots (8 and 4 days prior to potato and the same time with potato emergence in 2004 and the same time to potato, 2 weeks and 4 weeks after potato in 2005). For each year and location, primary tillage consisted of spring disking followed by field cultivation before planting. Potato was planted in constant density (5.33 plants per m^2) in May 26, 2004 and May 27, 2005. Weed emergence dates were recorded at the time of approximate 50% weed emergence. In the 3-4 leaf stage, weed seedlings thinned and field hand hoed to remove undesired weeds that had emerged. Two center rows used for data collection. Potato and Weeds were sampled at 2-week intervals. Light interception was recorded and analyzed with INTERCOM model. Daily solar radiation was obtained from Firoozkouh meteorological station. The daily PAR was assumed to be half of the daily global radiation. The absorbed PAR by species in mixed and pure stands was measured at 2 weeks intervals. The linear regression line between cumulative absorbed

PAR and cumulative dry matter were plotted for each plot. The slop is the radiation use efficiency (RUE).

Results and Discussion

Results of this investigation showed that by higher density of weeds, LAI and height of potato reduced and vertical leaf area distribution, LAD and light interception changed. By increasing weeds density and its interference, leaf area percentage in the second layer of canopy increased from 45 to 50 percent. Maximum LAI and LAD were obtained in upper layers of canopy. *Amaranthus retroflexus* and *Chenopodium album* reached to their LAD and maximum light interception in 55 to 80 cm of height in various treatments, while this point in potato was 40 cm. In other words, weed by putting more leaf are upon the potato canopy and more height and of course more light interception, can reduce potato yield. Results showed that by increasing weeds interference, light interception of potato was reduced. So, in 8 plant of lambsquarter per meter of row , potato light interception was reduced 43.3 and 53.5 percent in 2004 and 2005. In third level of redroot pigweed (8 plants per meter of row) reduction of potato light interception was 56.5 qnd 66.8 percent in 2004 and 2005, respectively. By 2 redroot pigweed per meter of row, RUE of potato increased 2.8 percent in comparison with control. It means redroot pigweed suppressed potato by shading. In the first and second levels of density of lambsquarter (2 and 4 plants per meter of row) results were similar. Average of potato RUE was 1.56 and 1.83 g/MJ intercepted PAR (for redroot pigweed treatment) and 1.72 and 1.89 g/MJ intercepted PAR in 2004 and 2005. On average, redroot pigweed and lambsquarter reduced RUE of potato by 10.5 and 4.6 percent.

Table 1- Change (%) in dry matter, absorbed PAR and RUE of potato in comparison with control in 2004.

Treatments		Change in comparison with control(%)					
		Dry Matter		Absorbed PAR		RUE	
		C. album	*A. retroflexus*	*C. album*	*A. retroflexus*	*C. album*	*A. retroflexus*
Weed Density	2 plants/m of row	-18.7	-21.8	-19.2	-22.1	+2.1	+2.8
	4 plants/m of row	-24.8	-39.7	-25.2	-25.2	+0.9	-19.4
	8 plants/m of row	-37.9	-47.3	-29.1	-24.9	-12.2	-30.3
Emergence time	8 days before potato	-36.3	-47.1	-19.8	-29.1	-19.2	-22.6
	4 days before potato	-30.4	-38.8	-17.3	-22.2	-14.1	-19.2
	Same time to potato	-22.3	-24.2	-14.9	-16.3	-7.9	-9.7

Table 2- Change (%) in dry matter, absorbed PAR and RUE of potato in comparison with control in 2005.

Treatments		Change in comparison with control(%)					
		Dry Matter		Absorbed PAR		RUE	
		C. album	*A. retroflexus*	*C. album*	*A. retroflexus*	*C. album*	*A. retroflexus*
Weed Density	2 plants/m of row	-12.9	-19.2	-15.3	-18.5	+2.6	-1.3
	4 plants/m of row	-19.5	-27.6	-20	-24.9	+0.5	-3.2
	8 plants/m of row	-26.5	-34.7	-22.7	-27.1	-5.4	-9.9
Emergence time	8 days before potato	-26.9	-35.2	-22.5	-27	-5.1	-10.8
	4 days before potato	-22.8	-25.1	-22.4	-24.9	-1.1	-3.2
	Same time to potato	-13.9	-18.9	-16	-18.5	+3.2	+0.4

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WATER SAVING, YIELD AND QUALITY CHARACTERISTICS IN EARLY POTATO CROP IN A MEDITERRANEAN ENVIRONMENT

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INTRODUCTION

The potato (*Solanum tuberosum* L.) is known to be sensitive to water deficit (Harris, 1978). To obtain high yields, in fact, the soil water content should not be lower than 50% of maximum available water in the root zone, especially during tuber formation. Even slight water stress causes a reduction in leaf size and photosynthesis, and consequently affects the number, size and the percentage of marketable tubers (Loon, 1981; Fabeiro et al., 2001).

In southern Italy, as in other Mediterranean coastal areas, the potato crop is grown in autumn-winter-spring cycle (from November-December to May-June) with the aim of obtaining an early product, which has a high economic value because it can be sold on foreign markets within the European Union (Foti, 1999). Potatoes grown for early production are also particularly sensitive to water stresses, which adversely influence not only tuber yield but also earliness (Foti et al., 1995; Mauromicale and Ierna, 1997; Ierna and Mauromicale, 2006).

Considering the decisive role of irrigation in early potato production and that irrigation water is an expensive and limited resource in the semi-arid areas of the Mediterranean basin, an important contribution may be provided by better irrigation management in turn leading to savings in water. A way of reducing water supply could be done by concentrating irrigation only in certain phases of the growing season.

The aim of the present work was to verify, under field conditions, how limiting water supply by irrigating only in some phases of the cycle affects both the productive and some qualitative characteristics in early potato crop.

MATERIALS AND METHODS

Experiment was conducted during 1998 at the experimental field of the U.O.S. of Catania, I.S.A.Fo.M. - CNR (National Research Council of Italy) on the coastal plain, south of Siracusa (37°03' N, 15° 18' E, 10 m a.s.l.). This is a typical area for off-season potato cultivation in Sicily. The climate is semi-arid Mediterranean, with mild winters, and commonly rainless springs. Frost occurrence is virtually unknown (two events in 30 years).

The soil type is calcixerollic xerochrepts (USDA, Soil Taxonomy), moderately deep; the moisture capacity was 29% of dry soil and the wilting point 11%.

In a randomized block design with four replications, using tubers of cv. Spunta four irrigation regimes: irrigation during whole cycle (A), irrigation up to the start of tuber formation (B), irrigation from tuber formation to the end of the cycle (C), irrigation up to plant emergence – dry control (D) were studied.

Irrigation was carried out over the envisaged periods to supply 100% ETC, calculated using the following formula:

$$ETC = \sum_{0}^{n} E\, Kc\, Kp$$

where: n= the number of days since the last watering; E=daily evaporation from an unscreened class A Pan situated about 40m from the crop; Kc= crop coefficient, which varied from 0.51 to 1.25 in relation to the phase of the crop's biological cycle; Kp=correction coefficient in relation to evaporation, which is 0.8 in the Mediterranean area (Doorembos and Kassam, 1979). Water was provided when the accumulated daily evaporation reached 30 mm by drip irrigation. Total amount of irrigation water supplied for different water regimes are reported in Table 1.

Whole tubers were planted manually on 20 January at a density of 4.76 plants per m^{-2}. All plants emerged 30 days after planting.

Table 1 - Total seasonal water inputs

Irrigation regimes	(m^3 ha^{-1})
A	1840
B	1080
C	1010
D	250

Ammonium mono phosphate (300 kg ha^{-1}) and chlorpyrifos (30 kg ha^{-1}) were applied before planting, and 200 kg ha^{-1} of ammonium nitrate after plant emergence. Standard crop management was applied, involving post-emergence weeding with linuron and pest control when needed.

Tuber yield measurement

Two harvests at 90 and 120 days after planting were made, in which for each plot tubers were hand harvested and weighed to determine fresh yield. The tubers were divided into waste (mean unit weight < 20 g) and marketable (mean unit weight > 20 g).

Quality characteristics

On a representative sample of tubers per plot at the end of the cycle (120 days after planting) the following were determined in the laboratory: dry matter content (thermoventilated oven at 105 °C), starch content (Fehling method by acid hydrolysis) and reduced sugars (Fehling method), protein content (Kjeldahl method) and ash (muffle kiln incineration at 600 °C) (Lotti and Galoppini, 1980).

Statistical analysis

Data were analyzed with ANOVA and means were separated on the basis of an *LSD* test.

Temperature and rainfall

Main meteorological data (minimum, maximum and mean air temperature, rainfall and evaporation), were recorded with a CR 21 data logger (Campbell Scientific, Inc., UK) located at the experimental site. As reported in Table 2, during the cycle (from January through May) the 1998 season, compared to that of the 30-year period, was associated with a lower than usual level of rainfall (145 *vs.* 186 mm).

Table 2 - Average maximum and minimum temperatures and precipitations for 1959-1988 and 1998

Meteorological variable	Year	Month				
		Jan.	Feb.	Mar.	Apr.	May
Maximum air temperature (°C)	1959-1988	16.2	15.3	17.0	19.8	25.0
	1998	16.6	15.8	17.5	17.3	23.5
Minimum air temperature (°C)	1959-1988	9.0	7.5	8.0	8.9	15.0
	1998	10.5	8.5	9.1	9.6	13.4
Precipitation (mm)	1959-1988	60	42	44	24	16
	1998	50	35	32	20	8

RESULTS

Water supply

Amounts of water supplied by irrigation including those to aid the rooting of the crop (250 m^3 ha^{-1}) are indicated in Table 1. Amounts of water supplied by irrigation in the regimes that envisaged irrigation during the first or the second half of the cycle (irrigation regimes B and C) proved similar and equal on average to 57% of that related to the regime with irrigation during the whole cycle.

Earliness and yield

Earliness, which is expressed by the amount of tubers obtained at 90 days after planting is more than doubled passing from the dry control to the tests foreseeing irrigation during whole cycle (from 22.4 to 47.6 t ha^{-1}); however, irrigation scheduled only for the first half of the cycle enabled a production of 44.4 t ha^{-1}, which is not significantly different to the irrigation during whole cycle (Table 3).

The effects of the water regime were also evident on the yield at the end of the cycle.

The highest yield (59.4 t ha^{-1}) was obtained when the irrigation was carried out for the whole cycle; nevertheless, the crop made efficacious use of the irrigation during the first half of the cycle, which determined with respect to that during the whole cycle only a reduction of 10%, but not that carried out during the second half of the cycle, that led to a reduction of roughly 34%, largely owing to the major reduction in unit weight.

The production of waste (tubers with unit weight < 20 g) calculated as percentage of overall production was notably reduced with respect to the dry control (14%), by the test with irrigation during the second half of the cycle (10%) and again more so the test with irrigation during the first half of the cycle (5%) without notable differences concerning the test with irrigation during whole cycle (6%) (Table 3).

Table 3 - Different tuber production parameters in relation to irrigation regimes. Within each column different letters indicate significant differences for P<0.05.

Irrigation regimes	Earliness (t ha^{-1})	Yield (t ha^{-1})	N plant^{-1}	Mean weight (g)	Waste (% of total)
A	47.6 a	59.4 a	8.4 a	202.0 a	6.2 c
B	44.4 a	53.4 b	7.8 b	186.2 a	5.4 c
C	29.1 b	39.5 c	7.3 b	149.6 b	9.9 b
D	22.4 c	35.9 d	7.5 b	121.3 b	14.2 a

Quality characteristics of tubers

Tuber dry matter content, increased significantly passing from irrigation during the whole cycle (18.0% f.w.) to partial satisfaction during the first or second half until reaching 20.7% of the dry control (Table 4). The trend of starch content concurred with that of dry matter, in view of the fact it represents the main component of the tuber dry matter. The quantity of reduced sugars equal to 2.2 ‰ f.w. in the test irrigated throughout or during the first half of the cycle, increased notably passing to the test irrigated during the second half (2.6 ‰ f.w.), and from this to the dry control (2.8 ‰ f.w.). Protein and ash content dropped more or less constantly from the test irrigated throughout to that irrigated in the first or second half of tuber formation to the dry control (Table 4).

Table 4 - Quality characteristics of tubers in relation to irrigation regimes. Within each column different letters indicate significant differences for P<0.05.

Irrigation regimes	Dry matter (% f.w.)	Starch (% f.w.)	Red. sugar (‰ f.w.)	Proteins (% d.w.)	Ash (% d.w.)
A	18.0 d	11.9 d	2.2 c	9.0 a	6.1 a
B	18.7 c	12.6 c	2.2 c	8.8 a	5.9 b
C	19.6 b	13.8 b	2.6 b	8.3 b	5.8 bc
D	20.7 a	14.2 a	2.8 a	8.0 b	5.7 c

DISCUSSION AND CONCLUSIONS

Under the specific conditions in which the experiments were conducted, the crucial role of irrigation in determining good earliness and yield of potato was confirmed. However, the results have highlighted the possibility of reducing water supplied by about 40 % with respect to the complete satisfaction by irrigating in the first half of the cycle without significantly affecting earliness and tuber yield. Irrigation during the second half was less effective, leading to a significant decrease in tuber production due largely to reduction in tuber unit weight.

The irrigation regimes also greatly affected some alimentary characteristics of the tubers. Passing from the complete satisfaction of water to the unirrigated control the dry matter, starch and reduced sugar contents increased, whereas protein and ash decreased. The increase in reduced sugars confirms the theory following which the stressed plant seeks to produce energy matter that may easily be used in the "recovery" phase once the adverse conditions are over. The reduction in protein and ash content may be related to the lesser absorption of minerals, such as nitrogen, owing to the limited water availability in the soil.

In conclusion, although these results are promising in that they may allow a notable saving in valuable irrigation water in the Mediterranean environment, they refer to only one year and one variety alone. Therefore, further investigations will be useful to extend this analysis to other cultivars that are already grown or may become widespread in the southern regions.

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USE OF SPAD CLOROPHYLL MEASUREMENTS ON OPTIMATION OF NITROGEN FERTILIZATION ON POTATO

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INTRODUCTION

Due to stronger demands on environmentally sounder production the attention is also paid increasingly on better utilization of fertilizer nitrogen on potato. In Finland studies do not support the split application of fertilizer nitrogen on potato as a standard method when the nitrogen is used according to recommendations (Kuisma 2002). On the other hand recommendations of fertilizer nitrogen on potato are so low in Finland that potato crop can suffer scarcity of nitrogen during the bulking if the mobilization of nitrogen resources in soil is restricted e.g. by cool or dry weather.

MATERIAL AND METHODS

In this study nitrogen status on potato crop was assessed using internal calibration with nitrogen windows described by Peltonen (2000). Zero windows and over fertilized windows were established on farm scale cultivations, which were given fertilizer nitrogen according to general recommendations. On zero-window no nitrogen was used and on over fertilized windows the nitrogen fertilization was doubled to the recommended use. The nitrogen of potato crop was measured non-invasively with the Chlorophyll Meter Minolta SPAD–502 (Konica Minolta Sensing Inc.) as averages of about 20 separate individual readings of distal leaflets on the youngest fully expanded leaves. Measurements were done once a week during 1–12 weeks after the emergence. The need of additional fertilizer nitrogen was calculated with the formula introduced by Peltonen (2000):

$$Need\ of\ additional\ N\ (kg/ha) = [N_{nw}/(SPAD_{nw}–SPAD_{nc})]x(0.95xSPAD_{nw}–SPAD_{nc})$$

where: N_{nw} = difference of nitrogen fertilization between over fertilized nitrogen window and the normally fertilized field
$SPAD_{nw}$ = SPAD reading of an over fertilized nitrogen window
$SPAD_{nc}$ = SPAD reading of the normally fertilized field

In 2001–2003 trials were located in Potato Research Institute at Lammi (61.1°N, 25°E) on two starch potato cultivars and on three, two and one cultivars of ware potato in 2001, 2002 and 2003, respectively. In 2004 trials were only on starch potato cultivations, two trials in the province of Satakunta at Kokemäki (61.2°N, 22.3°E) and Köyliö (61.1°N, 22.3°E) and three trials in the province of South Ostrobothnia at Alähärmä (63.2°, 22.5°E).

RESULTS AND DISCUSSION

Gianquinto et al. (2004) report that the useful period for nitrogen supplement lasts for 40–45 days after emergence at the start of full blooming. Similarly as Vos and Bom (1993) resulted, SPAD readings during the first month after emergence showed need of additional nitrogen fertilization only on unfertilized windows, irrespective of trial year, location or variety. SPAD readings of over fertilized windows exceeded values of standard fertilized potato crop not until six weeks after emergence and they stayed on this higher level near the end of measurements. The estimated need of nitrogen supplement on 0-windows was some over 70 kg/ha between 4–11 weeks after emergence on the average.

The nitrogen status of potato foliage on over fertilized windows proposed application of supplementary fertilization on normally fertilized potato crop between 6–11 weeks after emergence.

Need of extra nitrogen was about 15 kg/ha on the average. In the beginning of this period the

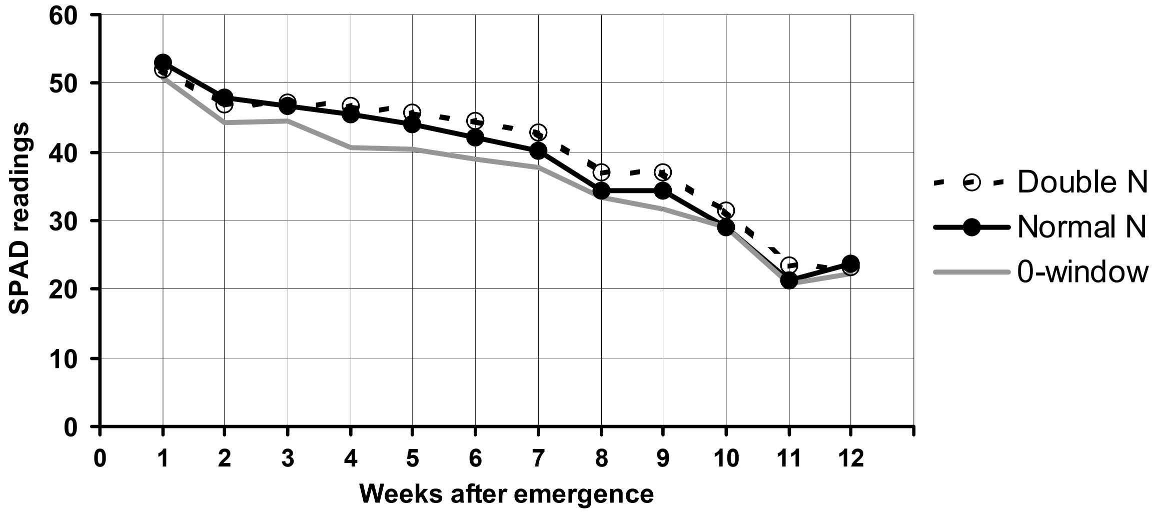

Figure 1. Development of SPAD readings on nitrogen windows

potato was already near the end of full blooming thus need of supplementary nitrogen was real only in 6–7 weeks after emergence. This conclusion was also supported by the final yields where only significant differences were detected between N-0 and fertilized crop.

The study showed that a hand-held chlorophyll meter SPAD is a usable tool to assess nitrogen status of potato foliage in the Finnish surrounding, too. With nitrogen windows it is also a suitable tool to decision support systems on optimation of nitrogen fertilization on potato in different surrounding and different types of varieties.

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HERBICIDES USED FOR WEEDS IN POTATO

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ABSTRACT

Weeds can reduce potato yield and quality by competing for light, water, nutrients and by interfering with harvest operations. Developing an effective weed control program potatoes requires careful consideration of such factors as the weed species present in the field, soil characteristics, tillage and irrigation practices, and crop rotation.

Herbicide management is a real problem to maintain the potato crop free of weeds.

Field experiments were carried out in the years 2005-2007 in Brasov and Targu-Secuiesc area to study the effect of some herbicides.

There are presented the yield variation in plots with and without herbicides.

INTRODUCTION

Potato is a crop with high sensitivity for weeds.

Weeds can reduce potato yield and quality by competing for light, water, nutrients and by interfering with harvest operations. Developing an effective weed control program potatoes requires careful consideration of such factors as the weed species present in the field, soil characteristics, tillage and irrigation practices, and crop rotation.

A combination of cultural practices and appropriate herbicides usually gives the most effective weed control Herbicide rates must be adjusted for soil texture, percentage organic matter, soil pH, weed species, potential for soil residue, and other herbicides used. (Georgeta Frâncu, 1988)

Weeds can be considered as dangerous as pests reducing yields. They are representing "the green pollution" (Berca M., 1998)

The field trials were established in the National Institute of Research and Development for Potato and Sugar Beet – Brasov and in Research and Development Station for Potato- Targu Secuiesc in 2005, 2006 and 2007.

MATERIAL AND METHODS

In Brasov area the soil is chernozem cambic type with 27% clay, 4.68 % organic matter and pH = 6.7 In Targu Secuiesc area the soil is chernozem rendzinic litic type with 36% clay, 3.8% organic matter and pH = 7.2

Potato used in Brasov was Sante variety, planted in 16th May 2005, 5 th May 2006 and 24th April 2007 and Productiv variety in Targu-Secuiesc planted in 5th May 2005, 2 nd May 2006 and 13 th April 2007.

Trials were carried out in 4 replicates plots in a randomised complete block, 5 rows each with 20 plants.

In Brasov all tested herbicides were applied in 6th June 2005, in 1st June 2006 and in 19th May 2007 and in Targu-Secuiesc the herbicides were applied in 1st June 2005, 25th May 2006 and in3th May 2007 .

The year 2005 was an unprecedented climatic year, with a lot of rain and high temperatures all the growing potatoes season.

Year 2006 was a climatic normal year, however in the first part of vegetation was registered a humidity deficit, the situation became normal in August.

Year 2007 was a very dry one till end of August, even in this conditions the soil capillarity give a good effect for the herbicides.

We harvested in 9 September 2005, in 20 September 2006 and in 3 October 2007. Yields were taken from two middle rows from each plot.

Table 1. Variants of pre-emergent herbicides treatment

Year	Product	Active ingredient	Dose
2005	Untreated (control)	-	-
	Sencor SC 700	Metribuzin	0,7-1,2 l/ha
	Boxer 80 EC	Prosulfocarb	3-5 l/ha
	Linuron 50% SC	Linuron	2-4 l/ha
2006	Untreated (control)	-	-
	Sencor SC 700	Metribuzin	0,7-1,2 l/ha
	Boxer 80 EC	Prosulfocarb	3-5 l/ha
	AS Super	Metribuzin	0,7-1,2 kg/ha
2007	Untreated (control)	-	-
	Sencor SC 700	Metribuzin	0,7-1,2 l/ha
	Stomp 330 EC	Pendimethalin	5,0 l/ha

RESULTS AND DISCUSSION

The weeds spectrum in 2005 to potato field: Galinsoga parviflora, Setaria glauca, Echinocloa cruss-galli, Polygonum lapatifolium, Veronica hederifolia, Convolvulus arvensis, Thlaspi arvense.

The weeds spectrum in 2006 to potato field: Setaria glauca, Galeopsis tetrahit, Convolvulus arvensis, Veronica hederifolia, Viola arvensis, Chenopodium album.

The weeds spectrum in 2007 to potato field: Setaria glauca, Echinocloa cruss- galli, Convolvulus arvensis*, Chenopodium sp., Cirsium arvens, Agropyron repens.*

Table 2. Weight of weed fresh matter/m² and dry matter 2005

weeds/ herbicide	Total weeds-Brasov		Total weeds-Tg. Secuiesc	
	Weight of fresh matter in g/m²	Dry matter in g	Weight of fresh matter in g/m²	Dry matter in g
Control	370.7	67.8	420.5	80.6
Sencor	5.7	1.9	11.5	5.8
Boxer	208.9	45.6	174.6	52.3
Linuron	36.2	12.8	78.9	54.2

Table 3. Weight of weed fresh matter/m² and dry matter 2006

weeds/ herbicide	Total weeds-Brasov		Total weeds-Tg. Secuiesc	
	Weight of fresh matter in g/m²	Dry matter in g	Weight of fresh matter in g/m²	Dry matter in g
Control	24.9	9.6	41.0	22.5
Sencor SC 700	10.2	3.8	12.5	4.2
Boxer	16.0	7.2	15.5	7.5
AS Super	16.4	7.0	15.5	7.5

Table 4. Weight of weed fresh matter/m² and dry matter 2007

weeds/ herbicide	Total weeds-Brasov		Total weeds-Tg. Secuiesc	
	Weight of fresh matter in g/m²	Dry matter in g	Weight of fresh matter in g/m²	Dry matter in g
Control	10.5	4.0	14.5	8.2
Sencor SC 700	4.9	2.5	5.8	2.7
Stomp 330 EC	5.5	3.4	6.3	4.2

Weight of weed fresh matter was 370.7 g/m² to the untreated variant in 2005 and 24.9 g/m² in 2006 and only 10.5 in 2007 to the same variant. An explication regarding the different weed infestation intensity is possible as result of soil weed reserve different from a parcel to other. Another explanation consists in the rainfalls and high temperature recorded all the season 2005.

Intensity of weed infestation was higher in 2005 than in 2006 and 2007.

Efficacy of Sencor (herbicide used in all years) was significantly high in 2007 and ranged between 98.5% and 99.7% in total weed-killing effect.

Weed infestation had statistically significant effect on reduce yield in all the years.

In 2005 the tuber yield in untreated plot was 68.3% comparative with Sencor variant, in 2006 was 61.6% compared with the same product and in 2007 was 84.8%.

Table 4. Tuber yield 2005-2007

herbicide	tuber yield t/ha 2005		tuber yield t/ha 2006		tuber yield t/ha 2007	
	Brasov	Tg. Secuiesc	Brasov	Tg. Secuiesc	Brasov	Tg. Secuiesc
Untreated	18.40	17.00	25.75	22.00	22.30	20.50
Sencor	26.93	24.40	41.80	35.50	26.70	25.20
Boxer 80 EC	25.0	22.20	-	-	-	-
Linuron 50% SC	24.50	24.00	-	-	-	-
Wizard	-	-	37.87	33.50	-	-
As Super	-	-	34.45	32.50	-	-
Stomp 330EC	-	-	-	-	26.30	24.50
DL 5%	5.19	5.0	6.12	4.5	4.6	6.19

CONCLUSIONS

Field trials conducted to INCDCSZ Braşov and SCDC Targu-Secuiesc during 2005-2007 studied the effect of some preemergent herbicides on weed control.

It's important to take account of the potential weed reserve in soil and to see the correlation between meteorological data and the intensity of weed infestation.

Also it's important to apply a graminicide when grasses have 10-15 cm.

Fall application of glyphosate is recomanded after the previous crop is harvested, to get a complete kill of the entire perennial weed system.

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THE INFLUENCE OF SELENIUM FERTILIZATION ON LEAF AREA INDEX (LAI), YIELD AND QUALITY OF POTATOES

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Selenium (Se) is a trace element that can function both, as an essential nutrient and as environmental toxicant (Fan et al., 2002) Unnecessary daily intake of selenium by humans should range 60-150μg Se. Negative effects of selenium occur when the intake is lower than 20μg $Se.day^{-1}$. On the contrary higher uptake (over 5mg $Se.kg^{-1}$ of food) is causation of selenotoxicosis of humans and animals (Trebichavsky, 1997). The narrow margin between beneficial and harmful levels of Se has important implications for human health. Plants can play a pivotal role in this respect. For example, plants, that accumulate Se may be useful as "Se-delivery system" (in forage or crops) to supplement the mammalian diet in many areas that are deficient in Se. On the other hand, the abilities of plants to absorb and sequester Se can also be harnessed to manage environmental Se contamination by phytoremediation (Terry et al., 2000).

Selenium ranks among antioxidants protecting against oxidative stress, together with vitamins A, C, E, beta-carotene and enzymes catalase and superoxiddismutase. Besides, Se is able to reduce adverse effects of smoking, protect organisms from progeria, and also participate in reinforcement of immunity systems (Paeffgen, 2004). Lack of selenium significantly increase probability of cardiovascular diseases and great deal of cancerous diseases. The probability increases in conjunction with decreasing concentration of selenium in organism (Kvicala, 2003).

Soils in central and north Europe are deficient in selenium content, this fact causes latent lack of this important microelement in whole food chain (Paeffgen, 2004). Almost half of Czech Republic population has the concentration 20-55μg $Se.l^{-1}$ in blood serum. This range signifies hard heavy deficiency with higher risk of diseases dependent on oxidative load (Kvicala, 2003).

The selenium content in potato tuber ranges 0.048–0.458 mg $Se.kg^{-1}$ dry weight in the Czech Republic (Koutnik, 1996) Foliar application of selenium was proven to be a good way to increase the selenium content of potatoes (Poggi, 2000). The tuber yield of Se-treated potato plants is higher and composed of relatively few but large tubers (Turakainen, 2004). Se leads to a reduction in mass of tubers in well-watered plants. Higher yield is reached by plants exposed to Se and limited supply of water (Germ, 2007)

The aim of our research was determination of optimal dose and form of selenium application, so that treatment would positively influence Se-concentration in tubers. In higher concentrations has selenium toxic effects and causes growth retardation, so it is important to set the dose, whose application would not markedly decrease potato yield-forming components.

The main benefit of this research should be increase of Se-concentration in potatoes, so that consumption of these potatoes with higher nutritional quality could contribute to increase of present insufficient intake of selenium by humans. On this account we also observed, whether and eventually how is affected selenium content in potatoes by culinary treatment i.e. boiling and frying.

Material and methods

Filed trials were established in the locality Zabcice in years 2006 a 2007. Potatoes were planted in 0.75 x 0.25m spacing on beginning of April, in both years. We used two different varieties; the early variety Karin and semi-early variety Ditta. In four repetitions we established control variant, two variants with Se-application to the soil before planting (doses 12 and 24 $kg.ha^{-1}$) and two variants with foliar application of Se (doses 200 and 400 $g.ha^{-1}$). Foliar applications were made on beginning of butonisation phase (dose 400g was divided in two doses, 200g was applied on begging of butonisation phase and next 200g one week later). In all variants we used the solution of sodium selenite. During vegetation in three terms (approx. after 60, 67 and 75 days of vegetation) was observed fresh weight of leaves, from which was consequently set leaf area index (LAI) by method of conversion rates (Zrust, 1974). Samples to be used for yield analyses were taken at the stage of table maturity, i.e. at the time the potatoes get to the consumer. In concrete terms, in both experimental years the varieties Karin and Ditta were sampled after approx. 90 (Karin) and 97 (Ditta) days of vegetation, respectively. In this analyses we observed yield of tubers per hectare and number of tubers per hill.

In same terms were taken samples for chemical analyses. Samples were processed in the usual way – rinsed in clean water (drinkable), peeled, cut into slices, dried and homogenised. One part of the tubers was processed in the standard way into chips and part was boiled. Both products were also dried and prior to analysis homogenised by grinding. After homogenisation the samples were decomposed in a mixture of HNO_3 and H_2O_2 in the microwave equipment MILESTONE MLS 1200 MEGA. After conversion to the defined volume the sample was analysed on the UNICAM 939 atomic absorption spectrophotometer using the method of production of hybrids by means of the UNICAM VP 90 vapour system. Selenium was calibrated to the certified calibration solution CZ 9051. The entire procedure was verified on certified reference material of CRM 402 white clover (Belgium).

Results

Leaf area index (LAI)

Results of leaf area index development are in Tab. 1. Statistically significant influence of year was found. In all three terms was higher LAI in year 2006. Significant differences between selenium treatments ware observed only in second term, when significantly lower LAI was observed in variants with soil application of Se in comparison with control variant.

Table 1: Leaf area index

		60 days		67 days		75 days	
		2006	**2007**	**2006**	**2007**	**2006**	**2007**
Selenium treatment per hectare	**Control**	3.203	1.019	2.894	1.901	3.593	2.792
	12 Kg	2.809	0.857	2.241	1.489	2.844	2.716
	24 Kg	1.966	0.933	2.524	1.321	3.149	2.757
	200 g	2.595	1.153	2.431	1.837	3.090	2.903
	400 g	2.536	1.199	3.044	1.634	3.587	2.336

Number of tubers per hill

Number of tubers was significantly affected by year, selenium treatment and also by variety. Higher number of tubers 13.51 $pcs.hill^{-1}$ was observed in year 2006, in 2007 it was only 5.73 $pcs.hill^{-1}$.

Higher number of tubers, 10.78 $pcs.hill^{-1}$, was reached by variety Ditta, whereas by Karin it was only 8.45 $pcs.hill^{-1}$.

In comparison of selenium treatments (Fig. 1) we found out, that application of Se to the soil (doses 12 and 24 $kg.ha^{-1}$) significantly decrease number of tubers compared to control variant. Lower number of tubers than control variant was observed also in variants with foliar application of Se, but the differences were not significant.

Yield of tubers per hectare

Hectare yields was affected by all three factors (year, Se-treatment and variety). In 2006 was observed mean yield 32.82 $t.ha^{-1}$, which is approx. two times higher than in 2007 (16.19 $t.ha^{-1}$). In comparison of varieties reached significantly higher hectare yield variety Ditta, concretely 27.61 $t.ha^{-1}$, which is 6.22 $t.ha^{-1}$ higher than mean yield, reached by variety Karin (21.,39 $t.ha^{-1}$)

When we compared Se-treatments, we found out, that all variants with applied Se reached significantly lower yield than control variant. There are also apparent differences between forms of Se-application. Soil application reduced yield about 35-40%, in comparison with control. Whereas in variants with foliar application was reduction of yield only within 20-25%.

Fig 1: Influence of Se-treatment on number of tubers per (pcs.hill^{-1})

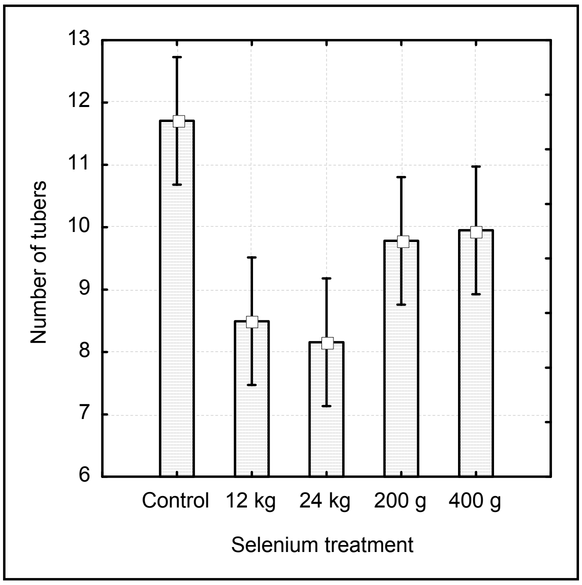
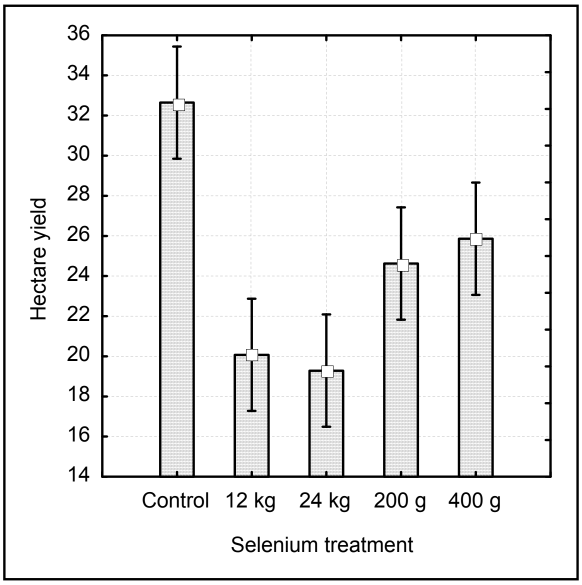


Fig 2: Number of Se-treatment on hectare yield (t.ha^{-1})



Selenium concentration in tubers

Content of selenium in tubers was significantly affected by year and Se-treatment. In the year 2006 was mean content of Se in tubers almost two times higher than in 2007. Concretely in the year 2006 was Se content in raw tubers 1.544 mg.kg^{-1} dry weight and in the year 2007 0.861 mg.kg^{-1} dry weight.

Concentration of Se in tubers increased with increasing dose of selenium (Fig. 3). Statistically significantly higher concentration was found in tubers from both variants with soil application of Se (i.e. variants 12 and 24 kg Se.ha^{-1}). Variant with applied 24 kg Se.ha^{-1} also reached significantly higher Se-content than both foliar variants (i.e. variants 200 and 400g Se.ha^{-1}).

Influence of culinary treatment

Culinary treatment of potatoes, it means boiling and frying, decreased the concentration of selenium (Fig. 4). Decrease of selenium content during boiling was 5 % in comparison to Se-content in raw tubers. Decrease of Se-content in fried chips was approx. 22%. But differences between any of analysed potato products (raw tubers, boiled potatoes and fried chips) were not statistically significant.

Fig 3: Selenium content in raw tubers (mg.kg^{-1} dry weight)

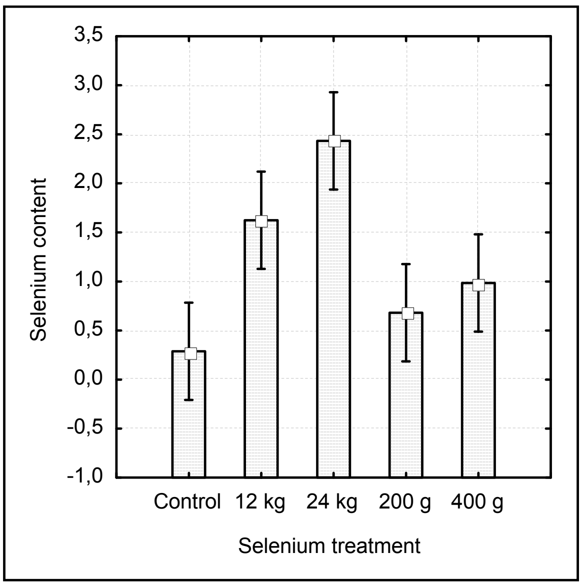
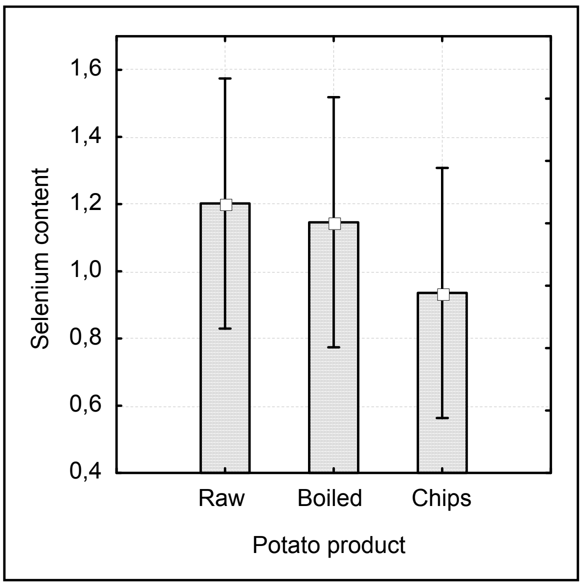


Fig 4: Selenium content in potato products (mg.kg^{-1} dry weight.)



Conclusions and discussion

On the basis of the results we can conclude, that almost all of observed characteristics were affected by year. This was caused by extremely dry weather in the year 2007, when during first two months of vegetation (April - May) were rainfall totals on locality only 29.2 mm, which is approx. one third of usually rainfalls in these two months.

We found out, that Se-fertilization inhibited development of leaf area during vegetation though differences were not statistically significant. This fact consequently caused decrease of hectare yields and number of tubers. Yield decreased with increasing dose of selenium. Higher decrease of yield was observed in variants, where selenium was applied to the soil. Our results differ from the results of Turakainen (2004) and Germ (2007). It is probably because we used much more higher doses of selenium than these authors.

Further, we found out, that Se-content in tubers increased with applied dose of selenium, withal higher concentration of Se was observed in variants with soil application of Se. But marked increase was observed also in variants with foliar application of Se. We achieved almost the same results as Poggi (2000), according whom are foliar applications good way, how to increase selenium content in potatoes.

Withal we determine positive fact, that during culinary treatment of potatoes decreases Se-content, but this decrease is quite low. Boiling decreased content of Se within 5% and frying within 22%. So we can claim, that sufficient amount of Se remains in potato products after culinary treatment. These "enriched potatoes" with a higher content of selenium could improve present insufficient intake of selenium in human nutrition and consequently they should become one of the preventive measures against cardiovascular and oncological diseases, to which a deficit amount of this essential element may contribute.

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POTATO CROP ESTABLISHMENT WITH USING OF LOCAL APPLICATION OF MINERAL FERTILIZERS

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Abstract

Local application of mineral fertilizers at planting is a perspective way of more efficient nutrient utilization. It is especially suitable in de-stoning technology, where higher nitrogen losses could occur due to increasing mineralization and nitrification processes. This fertilization system has been evaluated in the trials of PRI Havlíčkův Brod in recent years. A local application of solid mineral nitrogen fertilizers (ammonium sulphate) and liquid mineral nitrogen fertilizers (DAM 390) has been studied. The local application particularly increased tuber yield. In general, the results confirmed that this way of mineral fertilizer application is a suitable solution for the de-stoning technology.
Key words: potato, fertilization, nitrogen, yield

Introduction

De-stoning technology prior to potato planting affects physical soil characteristics. A positive effect of the technology was confirmed on soil compaction, clod presence, volume weight and porosity. However, an effect on soil moisture characteristics (maximal capillary water capacity, actual soil moisture and relative soil moisture) is less favourable. We can say that soil is loose through the whole vegetation, but is has higher tendency to higher drying. In general, the technology has very positive influence on tuber yield and mechanical tuber damage (Čepl, Kasal, 1999).

Fér (1995) refers that medium and smaller stones and clods are placed onto the bottom of furrows during soil separation and form a drainage layer there. Excess water runoff in the surrounding of placed stones has a favourable effect on erosion limitation; however in drier years it could be negatively expressed in faster runoff of rainfall water and deterioration of soil moisture conditions (Diviš, 2000).

Considering better soil loosening in de-stoning technology and faster soil warming, higher intensity of mineralization and nitrification processes occurs. Potato growing brings a relatively high risk of nitrogen losses (Neeteson, 1995) and it was found that gaseous losses of nitrogen could be also important in potato growing (Ruser et al., 1998). High nitrogen content in the soil, especially nitrate nitrogen, increases risk of nitrate leaching into surface and sub-surface waters before and during growing period and after the harvest (Haberle et al., 2002). As causes of this risk the authors mention several factors: potato growing in light soil and soil with pervious subsoil, locality with higher rainfalls or irrigated early potatoes, delayed onset of vegetation, high doses of nitrogen fertilizers, high content of soil nitrogen from autumn-applied manure, shallow to medium-deep potato root system, uneven water infiltration, high content of residual nitrogen after the harvest.

Maidl et al. (2002) describe an effect of application way and time on nitrogen utilization by potatoes using of 15N isotope-labeled ammonium nitrate Fertilization into the ridges at planting had a positive effect on tuber yielding ability, especially in single dose application at planting.

Local application is based on precise dosage and nutrient placement into root zone and this increases a possibility of fertilizer nutrient utilization by plants. The fertilizer is placed in the area of intensive rooting and therefore better utilization of supplied fertilizer amount could be expected. It could lead to higher yields and fertilizer savings (Pickny, Grocholl, 2003). Nutrient availability is also provided in dry period, when lack of soil moisture could hinder effective utilization of a fertilizer applied on the soil surface.

According to the results from Scotland in local application of a liquid fertilizer on average 13,1 % fertilizers (2-25 %) could be saved and ware potato yields are 16,6 % (10,6-20,6 %) higher.

Methods

In recent years we have evaluated a local application system of mineral fertilizers at planting in Potato Research Institute in Havlíčkův Brod. Classical broadcast application of granulated ammonium sulphate (AS) at rate of 110 kg.ha^{-1} was compared with local application of the same nitrogen level and about 30 % decreased level (80 kg N.ha^{-1}). We also established the same variants with the liquid fertilizer DAM 390 (DAM). Early ware potato variety DALI was used for the trial. In 2004-2005 tuber yield was the major studied index. Further, we also measured crop nutrient state in individual variants by a non-destructive method – chlorophyll content measurement (and/or intensity of green leaf colour) by N-tester.

Tab. 1: A scheme of trial variants with broadcast and local application of mineral fertilizers in 2004 and 2005

Broadcast application	Local application		Local application		Broadcast application
Ammonium sulphate	Amonnium sulphate	Ammonium sulphate	DAM 390	DAM 390	DAM 390
110 kgN.ha^{-1}	110 kgN.ha^{-1}	80 kgN.ha^{-1}	80 kgN.ha^{-1}	110 kgN.ha^{-1}	110 kgN.ha^{-1}

Results and discussion

Maidl et al. (2002) refer that an effect of locality and year is more important for tuber yield and plant nitrogen uptake than level of nitrogen fertilizers. A very strong effect of year was also expressed in our trials. Tuber yield in 2005 was almost 40 % higher compared to the year 2004 (Figs 1 and 2).

Results from 2004 indicate that yield amount in variants with local fertilizer application at planting with 10 % decreased nitrogen level almost correlated with yield level in the variants with full nitrogen doses in broadcasted mineral fertilizers. In variants with ammonium sulphate yield was by 9,7 % increased after local application of this fertilizer at planting in rate of 110 kg N.ha^{-1} compared to broadcast application of the same dose prior to planting. In case of the liquid fertilizer DAM 390 tuber yield was 17 % higher.

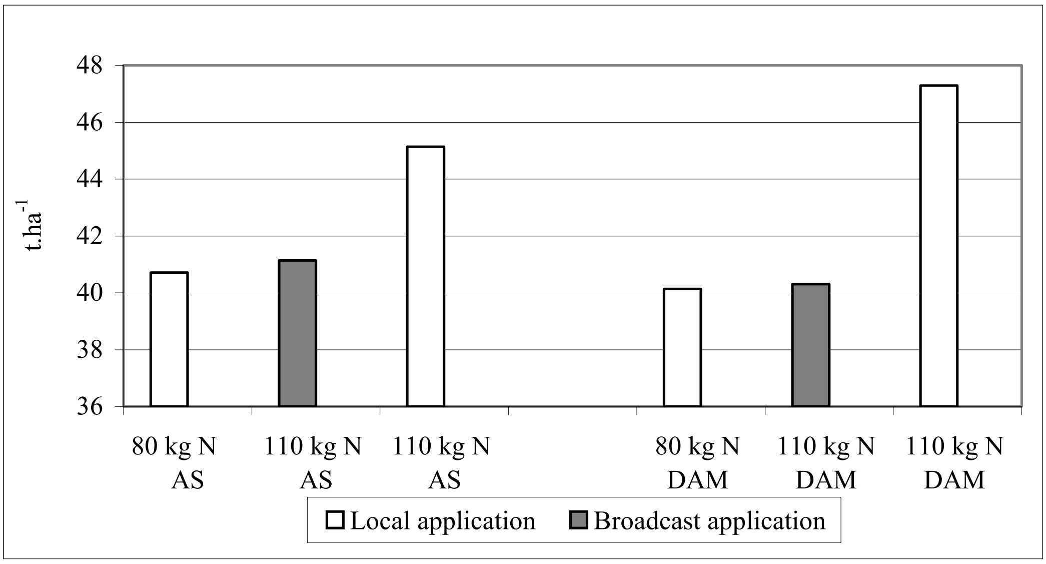

Fig. 1: Tuber yield (t.ha^{-1}) in 2004

In 2005 local application of 110 kg N.ha^{-1} provided 9,2 % higher yields in case of liquid fertilizer DAM 390 compared to broadcast application prior to planting. After local application of 80 kg N.ha^{-1} in DAM 390 tuber yield was 2,5 % lower than after broadcast application of 110 kg N.ha^{-1}.

Literal sources refer a possibility of yield increase or fertilizer rate decrease using of local application (Maidl et al., 2002; Pickny, Grocholl, 2003). Based on mentioned results we can conclude that efficiency of locally applied 80 kg N.ha^{-1} was comparable to broadcast application of 110 kg N.ha^{-1} in both years.

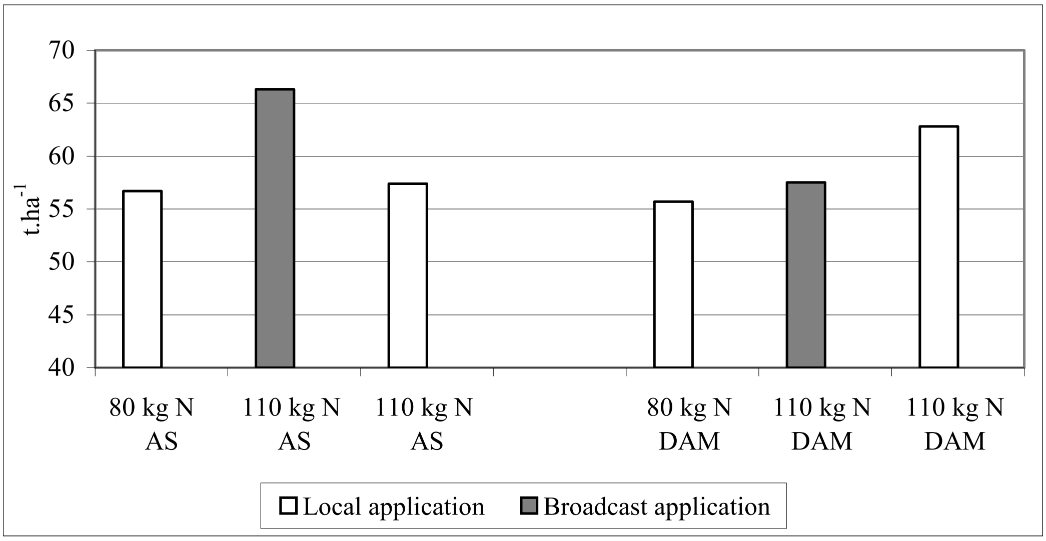

Fig. 2: Tuber yield (t.ha^{-1}) in 2005

Mean values derived from N-tester measurement in both trial years and their relation to mean values of tuber yield are presented in Figure 3. In case of DAM 390 we found more favourable plant nutritive state (higher values from N-tester) in local fertilizer application. Also mean value for yield of the variant with locally applied 80 kg N.ha^{-1} almost reached the yield of variant with broadcast application of 110 kg N.ha^{-1} (the difference was only 2 %). In variants with application of solid mineral fertilizer we measured the highest values by N-tester in the variant of local application of 80 kg N.ha^{-1} on average of both years.

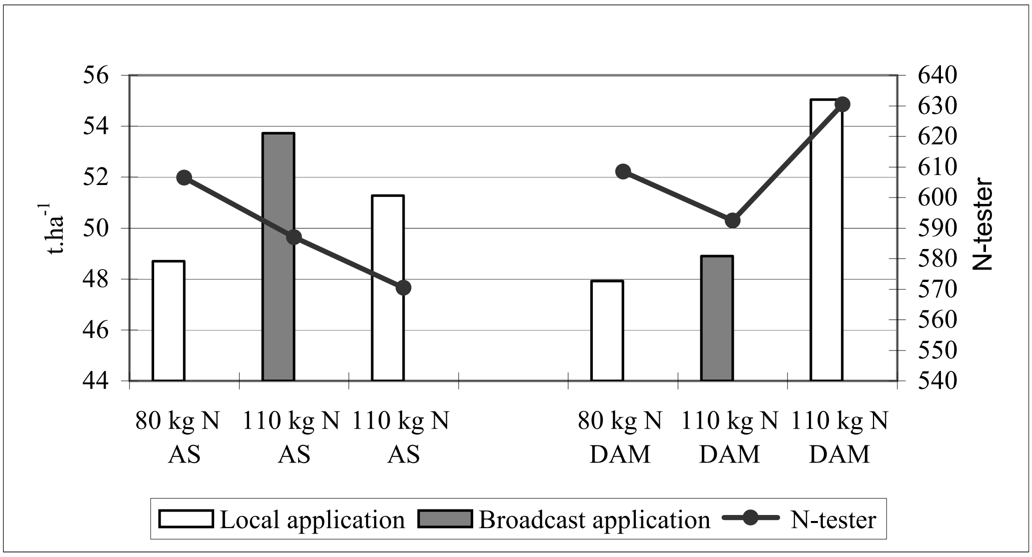

Fig. 3: Results of N-tester measurements in relation to tuber yields on average of the years 2004 and 2005

Conclusion

Results of the trials show that level of mineral nitrogen fertilizers could be reduced in local application and at the same time the yield level could be retained. Considering tuber yield an effect of broadcast nitrogen application in the rate of 110 kg.ha^{-1} was comparable to locally applied rate of 80 kg N.ha^{-1} using of liquid fertilizer DAM 390 in both years. In case of solid fertilizer ammonium sulphate, results of tuber yield were more favourable in broadcast application averaged over both years; this was affected by the results of 2005. Nitrogen rate of 110 kg.ha^{-1} compared to 80 kg.ha^{-1} increased tuber yields by 10 % averaged over years 2004 and 2005.

Acknowledgements

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IMPLEMENTATION OF A PILOT UNITY FOR POTATOES PRODUCTION

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Potato growers want to desert the field because there are problems concerning yield supply.

GENOTOXIC EFFECTS OF EXTRACTS FROM PESTICIDE-TREATED POTATO PLANTS

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Pesticides are extensively used to enhance an efficiency of agricultural productivity and crop yields. At present more than 1000 chemicals classified as pesticides and 2.5 million tons of pesticides are applied every year in agriculture world-wide (Torres et al., 1992; Dimitrov et al., 2006). Because large amounts of these chemicals are released into the environment and many of them affect non-target organisms, they represent a potential hazard to ecosystem health. Many studies have shown that majority of pesticides possess genotoxic properties inducing DNA damages, mutations, chromosomal aberrations (De Bertoldi, 1996). Experimental data revealed that pesticide residues can be present in vegetables and fruit and represent a risk for human exposure (Feretti et al., 2007).

In the present study we have evaluated the genotoxic effects of extracts of potato plants *Solanum tuberosm* L. exposed to the pesticides under laboratory conditions.

The following five pesticides were obtained from the Tatar Institute of Agriculture, Kazan, Russia. Sencor (metribuzin) is a selective triazine herbicide. Mospilan (acetamiprid) is an insecticide effective against a broad spectrum of agricultural insect pests. Pencozeb (mancozeb) is a fungicide. Actara (thiomethoxam) is a compound with a broad-spectrum insecticidal activity. Fastac (alfa-cypermetrin) is pyrethroid insecticide. All these pesticides are widely used for treatment of potato plant lands in Tatarstan, Russia.

Potato plants (80 for each compound tested) were grown in mineral medium (Negruk, 1989) containing one of tested pesticides (Sencor – 50 μg/ml, Mospilan – 50 μg/ml, Pencozeb – 5 μg/ml, Actara 50 μg/ml, Fastac –0.02 μl/ml) for 3 weeks. After exposure the potato leaves were collected and used for preparation of extracts. Water and DMSO plant extracts were tested for genotoxicity using Ames test. For all experiments we used *Salmonella typhimurium* auxotrophic tester strain TA 100 (*his G46, rfa,Δ uvrB, bio,*$^-$ *pKm 101*) kindly provided by Dr. B. Ames (Berkeley, USA). The tester strain can revert to histidine prototrophy (His$^+$) through specific gene mutations. The number of His$^+$ revertants colonies is a direct measure of the mutagenic potential of test substance. Water and DMSO extracts of control plants were used as a negative control. A plate incorporation test was performed according to the method described by Maron and Ames (1983).

The results presented Fig.1 indicate that water extracts of leaves from potato plants treated with pesticides Actara, Mospilan, Fastac, Pencozeb did not induce a marked increase in number of His$^+$ in Ames test. The weak mutagenic activity was found for water extracts of leaves of potato plants exposed to Sencor. We observed more than two-fold increase of induced of His$^+$ revertants values over the number of spontaneous revertants for *Salmonella typhimurium* TA 100.

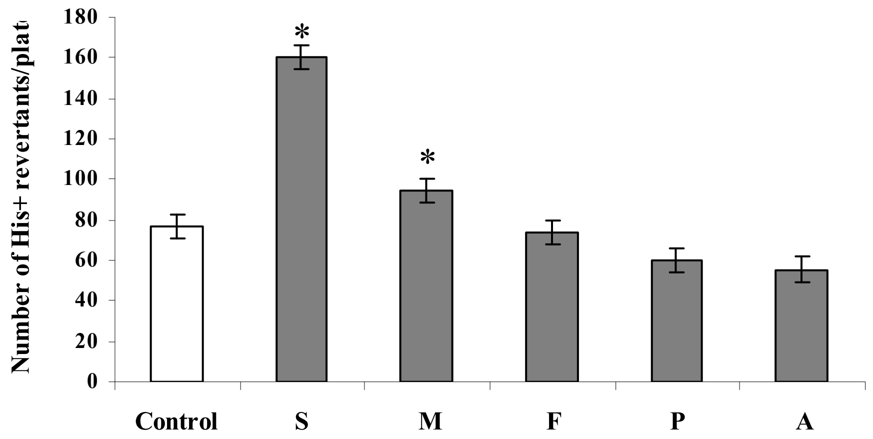

Fig.1 Mutagenic effect of water extracts of leaves from pesticide-treated potato plants. S-Sencor, M-Mospilan, F- Fastac, P – Pencozeb, A – Actara. Data are expressed as mean number of three experiments ± SE. * - Statistically significant compared with the control (P ≤ 0.05)

As seen from results presented in Fig.2 DMSO extracts from the potato plants treated with all the pesticides tested did not induce any genotoxic effect in *Salmonella typhimurium* strain TA 100.

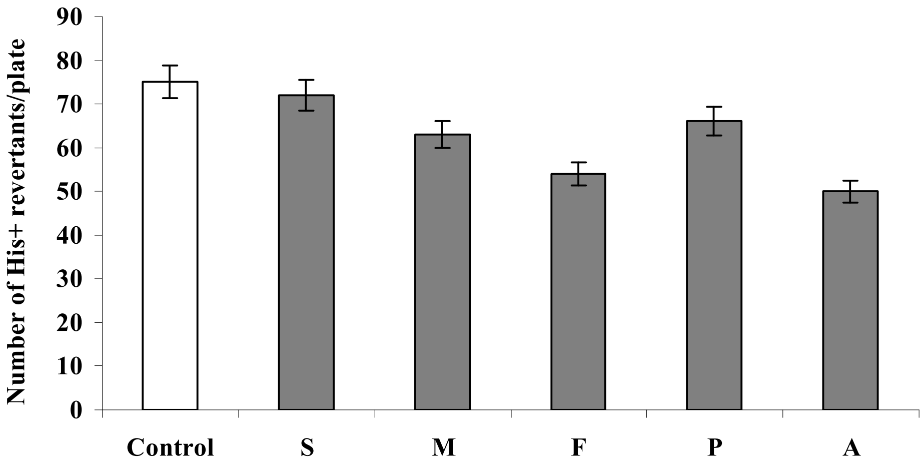

Fig.2 Mutagenic effect of DMSO extracts of leaves from pesticide-treated potato plants. S-Sencor, M-Mospilan, F- Fastac, P – Pencozeb, A – Actara. Data are expressed as mean number of three experiments ± SE.

In conclusion the data from our study indicate the necessity of more detailed investigation of herbicide Sencor genotoxicity taking into account the plant metabolism of this compound. Thus, in considering the accumulation of Sencor residues in plant tissues in dose inducing gene mutations much attention should be given to control this pesticide in the environment.

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ECOLOGICAL ASPECTS OF FERTILIZERS APPLICATION IN POTATO GROWING OF RUSSIAN FEDERATION

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The article is devoted to the results of the investigation of fertilizer and soil improver application for the recent 20 years. It was found out that the fertilizer dosing should be calculated taking into account the nutrient carry-over and soil diagnosis. It was the main reason for obtaining ecologically pure production and preventing nutrient losses during atmospheric precipitation infiltration. In sward-podzolic soils it is necessary to apply Ca, Mg and S besides the NPK macroelements. Calcium and magnesium carry-over with potato yields is twice as much as phosphorus one.

To compensate the negative influence of environmental stressors (such as short-term droughts or rain surplus) it is necessary to apply soil dressings or top ones. In 2006 foliar top dressings with chelate forms of Aquarine-12 microelements resulted in high potatoes yields. Top-dressing was carried out in accordance to the results of foliar diagnosis.

The green manure (such as sweetclover, lupin) increases fertility of sword-podzolic light-textured soil and productivity of potato. Green manure crops suppressed potato-root eelworm. Sweetclower ploughing resulted in 4.2 t/h increase in potato yield, and lupin one resulted in 3.7 t/h increase. The potatoes had the high content of dry matter, starch, ascorbic acid and a good taste after cooking if it had been grown on a background of green manure.

Grain straw is an alternative source of organic fertilizer. The optimal depth of its ploughing is 0-6 cm. Leguminous green manure crops are necessary to sow into the top mulching layer of the straw.

Key words: green manure crops (green manure), macroelements (N, P, K, Ca, Mg, S) and microelements (Zn, Cu, Mn, B) in chelate form, limy improvers.

Introduction

In the Russian agriculture there is an inadequate judgement about ecological conditions and an estimation of ecological consequences of chemization of an agriculture. It is strongly exaggerated aside costs of chemization. Certainly, the ecological risk of rash application of means of chemization exists.

However, rational application of fertilizers is not of considerable ecological danger to the environment. It is much worse if fertilizers are not applied or their doses are unreasonably small. The investigations, carried out during long-term stationary experiments, demonstrate that the process of soil fertility degradation, when fertilization is absent, proceeds much sooner than the cultivation because of application of economically substantiated doses. Even the supporters of organic farming admit the importance of fertilizers to supply the nutrient carry-over with the yields of crops.

So, for conditions of modern potato growing, the correct decision of environmental problems of chemical and pesticide application is to optimize their dozes, instead of the refuse or minimal application. Rational dozes of fertilizers, chemical and biological amelioration in aggregate with an optimal agrotechnics and the crop rotations adapted to soil-climatic conditions are responsible for maintenance of resistance and high efficiency of agrocenosises

The famous German scientist G. Kant, well known in the field of biological plant growing (1988), supports the reasonable usage of fertilizers and pesticides at a priority of biological agricultural methods. In accordance to his statement, the agrarian landscape only carries out its multipurpose role upon the condition if the agriculture will be "integrated", "sparing" and include the positive features of alternative and intensive technologies.

Results and their discussion.

The potato presents great demands for nutrient presence in the soil .It requires the significant amount of nutritious elements for tuber yield producing within a rather short vegetative period .

As it was known, the nutrient carry-over by plants considerably changed depending on the level of yields. Nitrogen carry-over by potato was 100-135 kg/hectare, phosphorus - 24-27, potassium - 200-239, calcium - 29-36, magnesium - 8-9 and sulfurs 6-7 kg/hectare – in the variant without fertilizer application. Fertilizer application considerably increased the carry-over of nutrients: the increase was 2-fold for nitrogen, 1.5-fold for phosphorus, 1.9-fold for potassium, 1.6-1.7 fold for calcium, magnesium and sulfur.

During the period of the investigation the maximal potato productivity and consequently the maximal nutrient carry-over were observed on a background of a full doze of dolomitic flour (1,0 t /h.) together with some fertilizers. In these variants with the tuber yield of 35-40 t/h (without the haulm), it was annually irretrievably lost 100-130 kg of nitrogen, 22-26 kg of phosphorus, 200-230 kg of potassium, 9-10 kg of calcium, 11-15 kg of magnesium and 3 kg of sulfur.

However biological nutrient carry-over (considering the haulm) was doubled when the heavy potato yields formed and it was for nitrogen -200-230 kg/h, for phosphorus - 33-37, for potassium -320-380, for calcium -45-50, magnesium -20-30 and for sulfurs -8-10 kg/hectare. The total carry-over of calcium and magnesium was 2 to-2.2 times as much as the one of phosphorus.

Worse losses of nutrients in potato plantings resulted from water infiltration during the lysimetric studying (for 10 years) the composition of infiltrative waters under potato plots. It was found out that maximal element losses were noticed in autumn after the harvest. They were 45-56 % of total yearly losses. The average annual losses of nutrients from a 40 cm layer of soil (0-40 cm) were: calcium - 55-127 kg/h, magnesium - 22-75 kg/h, nitrogen (nitrate) - 23-69, potassium - 6-26, sulfurs (S-SO_4^{2-}) - 43-105 and chlorine - 24-138 kg/h. Increasing dozes of fertilizers resulted in 1.5-2.6 fold increase in nitrogen leaching, 2.1-4.4 fold increase in potassium leaching, 1,5-4,4 fold increase in chlorine, 1.3-2.2 fold-in magnesium, 1.1-1.6 – in calcium and sulfur leaching comparing with the losses from the unfertilized soil.

Therefore, so that the soil was not exhausted due to washing away the nutrients with atmospheric precipitation and alienations with heavy yields, first of all, it was necessary to maintain and increase the fertility of arable lands.

Low parameters of soil fertility (high acidity, the low content of accessible nutritious elements, humus, etc.) are characteristic of the majority of arable soils in the Russian Federation. In this connection, mineral and organic fertilizer application is a necessary condition of high agriculture profitability. Dozes of fertilizers should be counted on the base of the balance methods taking into account soil diagnosis and a planned level of productivity. Application of fertilizers, considering soil-vegetative diagnosis, is one of the elements approaching us to the resource usage as though saving up agriculture.

In the Non-chernozem zone the immediate measure to improve the fertility of acid soils is liming. As it is known, the potato rather easily suffer soil acidity, the optimum reaction of the substrate for it is in a slightly acidic interval (pH 5.0-5.5). Optimal improver dozes (3-7 t/h of dolomitic flour by physical mass) allowed to save 30 % of nitric fertilizers. Liming is an energy-saving element in potato crop rotations.

However in practice, potato planting liming is often accompanied by spreading potato corky scab on the tubers. For this reason the large-scale soil liming in potato crop rotations is not carried out. In this connection, the breeder’s task is to enhance breeding potato cultivars resistant against potato corky scab besides the cultivars resistant against potato late blight and virus diseases.

Now it is possible to take a compromise decision - to replace the application of liming in potato crop rotations to a complex mineral nutrition, which besides nitrogen, phosphorus and potassium must include calcium, magnesium and sulfur. The potato strongly responds on magnesium, the amount of this element in the soil should be up to standard 33-49 mg/100 g of soil. Its application results in better development of tubers and root crops rather than haulm. Magnesium reception in plants is not only determined by presence of the available form in the nutrient substrate, but it also depends on its ratio to the other cations Considerable prevalence of calcium over magnesium and potassium, when high doses of chalk, lime and others calcium containing limy materials are applied - is a principal reason for the negative influence of liming using these materials. When potato is grown on light-textured soils, we very often come across the lack of magnesium and even magnesium deficiency which amplify when we apply heavy dozes of potassium fertilizers, and aluminium and manganese salts presence in the soil solution The more acid the soil, the heavier doze of magnesium fertilizers should be. In acid soils (pH <5), if the manure has not been applied, in spring it is annually advisable to supply all crops with soluble magnesium fertilizers (magnesium sulphate, potassic magnesium). In soils with the neutral reaction (pH = 6-7), magnesium content is more, so only magnesium demander crops(such as potato and beet) can be supplied with it at a rate of 30-40 kg /h MgO or 190-250 kg/h of magnesium sulphate by physical weight.

To compensate the negative influence of stressful climatic situations (short-term droughts or surplus of rains) which almost annually repeat in various zones of the country during vegetation and result in considerable shortages of tuber yield and the reduction of production quality, it is necessary to more widely apply root or foliar top dressings. In the experiments carried out in the conditions of Ryazan and Moscow regions, on grey wood and sword-podzolic medium textured loamy soils (Shestakov, 2006), it was found out that fertilizer application fractionally and locally - under milling cutting of crests at the doze of $N_{32} P_{26} K_{48} Mg_8 S_8$ and 2 weeks later, when hilling - at the dose of - $N_{21} P_{17} K_{32} Mg_5 S_5$, favoured forming heavy yields of potato which were characterized by quite high quality and preservation ability and the lowered content of nitrates . Fractional fertilization reduced negative loading on soil microflora at the moment of application and raised the coefficient of nutrient consumption both in rainy and drought years. According to A.N. Nebolsin (2003) in the conditions of rainy weather when N_{60} was applied, the nitrates concentration in leakage waters varied from 2 to 7 mg/l, and when N_{120} was applied in a lump, the nitrate concentration could reach and even exceed 8-17 mg/l, at a maximum concentration limit in drinking water of 10 mg/l.

Results of the experience, carried out in ARSRIPG (2007) in the condition of extremely droughty vegetation period of 2007 showed that double spraying with complex of microelements in chelates form (trade mark “Microvit”) in dose: 1.0+1.0 l/he in blooming (02.07.07) and in the end of blooming (18.07.07) were increased yield rate of middle early variety Nevskiy on 41-76%. Microelements improved metabolic process and potato ripening, finally concentration of non utilized nitrate decreased in variants with no rooted top-dressing of «Microvit» in 2 times, the content of ascorbic acid increased for 2,5-4,5 mg% in comparison with untreated control. On the variants with microelements top-dressing were obtained essential increase of valuable nutrients from square unit: dry matter for 6,6-11,4 centner/he; starch for 4,4-6,7 centner/he ;ascorbic acid for 7,4-13,0 centner/he in comparison with control. Integrated use of advanced achievements of the chemical industry allows to open more full biopotential of a potato at simultaneous decrease in dozes of pesticides.

Within the last decades the losses of humus in soils in the main agricultural zones of the country have reached the significant sizes. The reduction of humus content in the soil negatively affected on agrophysical characteristics. In the universal crop rotations the annual losses of humus were 500-1000 kg/hectare, and in the intertillage ones they reached 800-1500 kg/hectare. The application of 1 ton of well prepared manure left very little humus in the soil - 40-50 kg. That meant, that to complete stocks of humus in intertillage crop rotations, it was required to apply 16-30 t of manure per a hectare of arable land annually.

Traditional organic fertilizers by the force of the influence on productivity and the content of nutritious components in tubers do not concede the mineral ones. It is not effective to apply the manure and mineral fertilizers under potato in the same year because of sharp deterioration of production quality. It is desirable to bring the organic (at dozes of 40-60 t/hectare) under the previous culture, and the average dozes of fertilizers can be used directly at potato planting.

Combination of organic and mineral fertilizers (organic-mineral system of nutrition), alongside with a correct crop interchange in a crop rotation, promotes the activization of soil microflora which regulates the processes of nitrogen fixation and mineralization of organic soil substances.

Completing the stocks of soil organic substance should occur not only due to application of traditional organic fertilizers and composts on their basis, but also due to the expansion of green manure crops, perennial grasses, and introduction of highly productive bean cultures, introduction of the intermediate crops into rotations, rational use of grass straw.

The green manure is an alternative source of organic substance and biological nitrogen. It promotes mobilization phosphorus, potassume, calcium, magnesium, microelements from underlaying genetic horizons of soil and their involving in the biological circulation. Green manure crops reduce field weedness, carry out a fytosanitory role, improve water-physical properties of soil, increase the efficiency of a crop rotation and quality of obtained production.

In the stationary field experiment in ARSRIPG (2004-2007) on sword-podzolic sandy loam soil, the increase of potato yield was 4,2 t/h after sweetclover ploughing and it was 3.7 t/h-after lupin ploughing. The Top yield of potato was obtained after sweetclover ploughing on a background of liming with dolomitic flour (0,5; 0,75 h. ac.) and metallurgical slag at the doze of 0,5 h. ac. - 38,7-38,9 t/h. The potatoes had the high content of dry substances, starch, ascorbic acid and a nice taste after cooking if it had been grown on a background of green manure.

A cheap source of organic and inorganic substances is the straw of grain crops. It is economically profitable and ecologically safe to grind fine and to close up in the soil at the depth of 0-10cm the postharvest residues of grain predecessors - straw and a stubble, to provide their maximum decomposition before winter. Covering is carried out by two traces: the disks (if the field is weeded by annual weeds) or botton topsoil plows, chisel cultivators (for destruction of a couch grass and other rootstock weeds). At a deep straw ploughing (0-20 cm) some phytotoxic substances (H-hydrooxybenzoic, coumaric, salicylic, ferulic and other acids) are formed in the soil in the conditions close to anaerobic ones (Nicze L.K., 2005). One of the factors reducing depressing effect of the straw is to reduce the depth of its closing up in the soil. The straw makes the best effect on plant growth and development when it is applied in to the top layer of the soil (0-6 cm).

High grain yields (4-5 t/h) result in the increase of straw mass in the soil, the concentration of toxic substances increases and the depressive influence of straw enhances. To avoid it, it is necessary to create the best condition for organic substance decomposition. These conditions take place when we use green manure together with straw. This method is very suitable for the southern regions of this country. In the northern regions, when straw grinding, it is necessary to apply additional 10 kg of N per every ton of straw. When the straw is ploughed without previous additional nitrogen application, the nitrogen immobilization by the microbe biomass of soil takes place because of the low nitrogen content in the straw (C: N=60-100). This process lasts until the C: N ratio reach 20.

Conclusions.

Thus, the prospects of potato growing development in the Russian Federation can be provided as a result of practical realization of a whole complex of scientifically based agronomical methods, such as: integrated application of pesticides and balanced dozes of mineral fertilizers, that are based on the results of soil-vegetative monitoring accomplished on a background of maximum using biological factors, which improve the productivity of potato crop rotations. Application of agrochemicals and pesticides should be realized on taking into account the biological features of various potato cultivars when they are grown in the concrete soil-climatic region, observing the rules of application to keep up the ecological safety of environment.

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RESULTS OF USING DRIP IRRIGATION FOR GROWING POTATO IN SOUTH ROMANIA

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Abstract

Drip irrigation is a perspective way of spreading out the potato crop in South Romania, particularly on soils not so favorable for potato growth such as soils with high clay content. This irrigation method has a positive impact both on the soil and potato plant assuring high yields in areas where normally the potato is not suitable to be cultivated. Thus, there are farms in South Romania that are using drip irrigation systems and that are cultivating potato with good results, even on soils with high content of clay. In the present paper, there are presented some results regarding the potato drip irrigated, but also some effects of this irrigation method within the system soil – potato plant – crop technology – environment.

Key words: potato, drip irrigation, South Romania

Introduction

Drip irrigation is a method which consists in a slow delivery of water on the ground, drip by drip. Water is delivered punctually to the plants in a small debit and with an almost null pressure by the help of the capillary micro-tubes. Drip irrigation covers the water needs of the plants and assures a good water balance which is favorable for the growth and development of the potato plant. By determinations and measurements, it is possible to precisely establish the plant needs in water, and the drip irrigation method assures a strict control of using the water factor. On the other hand, drip irrigation is the most suitable irrigation method in dry areas [1] and makes possible the potato cultivation on not so good soils, such those with high content of clay.

Drip irrigation has some specific characteristics, such as:

- Drip irrigation has the possibility to have a good control of the irrigation norms and determines an important reduction of losses of water through the evaporation process.
- Also, drip irrigation eliminates the peripheral losses of water, such as irrigation of roads, neighborhoods, etc.
- This irrigation method needs very slow pressure of water, which means important energy savings and a safety utilization of the whole irrigation system.
- Drip irrigation is less depending on soil, relief, hydrologic and wind conditions.
- Through this irrigation method, the soil temperature is higher than through using other irrigation methods, which means the plants could reach maturity earlier.

Materials and Methods

In view to find out the specific effect of drip irrigation upon the potato cultivated on a soil with high content of clay, under the specific climatic conditions of 2006, there was carried out some research and studies on four potato varieties, Ilona, Futura, Dura and Rostara respectively. In this respect, there were organized two field experiments within the Experimental Farm Moara Domnească (15 km North-East far way from Bucharest) belonging to the University of Agronomical Sciences and Veterinary Medicine of Bucharest. Each experimental fields had a total surface of 0.2 ha, one of them being with drip irrigation and one without irrigation.

The irrigation norm was 200 m^3/ha, the number of irrigations was seven, which means a total norm of irrigation of 1400 m^3/ha.

In the year 2006, the average air temperature in the studying area was higher than the annually average on spring (except May), August and September, and was smaller than the annually average on May, June and July, which means that in the active growth period for potato the temperature was more favorable than in other years. The rainfall sum were higher than the annually average on April, August, and September, but in total there was a deficit of 20,4 mm for the first nine months compared to the annually average (figure 1).

The determinations were focused on the following: plant height, average weight of tuber, plant production and production per surface unit.

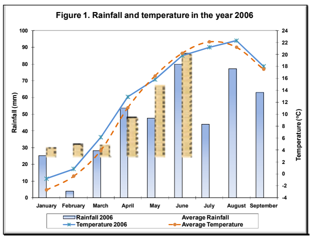

Figure 1. Rainfall and temperature in the year 2006

Results and Discussions

Analyzing in complex the effect of drip irrigation within the system soil – potato plant – crop technology – environment, one can remark that there are effects upon the potato plant, soil and crop technology, as follows [2]:

- Effects upon soil:
  - Drip irrigation avoids soil structure degradation, levigation of the nutrients, soil compaction and crust formation, which is particularly important for potato growing and tuber formation on soils with high clay content.
  - Drip irrigation avoids the risk of fungal and bacterial contamination through the splashing of potato plants with soil particle.
  - Soil could be maintained to an optimum and constant humidity level, according to the specific plant requirements for each growth and development stage.
- Effects upon plant:
  - Leaves are not wet during drip irrigation, which means there is a limitation of diseases favored by wet mediums.
  - Also, because there is no need to enter into the crop especially because of this irrigation method, there is limited spreading out of diseases.
  - Drip irrigation does not cold suddenly the epigeous organs of the potato plants, eliminating the restrictions related to the hour of irrigation, especially in the cases when there is high air temperature and water has low temperature.
  - Drip irrigation also does not cold suddenly the root system of the potato plant because of the regular and slow addition of water.
  - Potato roots have an adequate respiration process during the whole period of vegetation as the micro pores of the soil contain air, and just a small area around the trickle points is water saturated.
  - Water distribution is uniformly made in points closed to the potato plant, drip by drip, in a quantity and frequency adequate to the plant needs.
- Effects upon crop technology:
  - The functionality of the drip irrigation system does not depend on the other technological measures in the crop technology.
  - Also, the other technological measures in the crop technology could be made independently of the functionality of the drip irrigation system.

The average height of plant varied from 83.8 cm (Futura variety) to 87.2 cm (Dura variety), under drip irrigation conditions, while under no irrigation the average height of plant varied from 48.7 cm (Ilona variety) to 52.3 cm (Futura variety). For the four potato varieties, the average height of plant was 85.6 cm under drip irrigation conditions, while under no irrigation conditions the average height of plant was 52.4 cm (table 1).

The average weight of tuber varied from 106.5 g (Rostara variety) to 119.2 g (Ilona variety), under drip irrigation conditions, while under no irrigation the average weight of tuber varied from 47.5 g (Rostara variety) to 50.1 g (Ilona variety). For the three potato varieties, the average weight of tuber was of 112.2 g under drip irrigation conditions, while under no irrigation conditions the average weight of the tuber was of 48.8 g.

The average yield per plant was between 734.8 g (Rostara variety) and 834.4 g (Ilona variety), under drip irrigation conditions, and it was between 304.4 g (Dura variety) and 350.7 g (Ilona variety), under no irrigation conditions. For the three potato varieties, the average yield per plant was 782.1 g under drip irrigation conditions, while under no irrigation conditions the average yield per plant was 325.5 g.

The average yield per hectare was between 36,740 kg (Rostara variety) and 41,720 kg (Ilona variety), under drip irrigation conditions, and it was between 15,437 kg (Rostara variety) and 17,535 kg (Ilona variety), under no irrigation conditions. The average yield per hectare for the four studied varieties was 39,105 kg, under drip irrigation conditions, and it was 16,349 kg, under no irrigation conditions.

In average for the four potato varieties, under the climatic conditions of 2006 and on a soil with high content of clay, the drip irrigation determined compared with the no irrigation situation, a plus of 63.3% for the height of plant, a plus of 129.9% for the average weight of tuber, a plus of 140,2% for the average yield per plant and a plus of 139,1% for the average yield per hectare.

Table 1 The effect of drip irrigation in the potato varieties Ilona, Futura and Dura - Moara Domnească, 2006

Variety	Average heigh of plant (cm)		Average weight of tuber (g)		Average yield per plant (g)		Average yield per surface unit (kg/ha)	
	Drip irrigation	Without irrigation	Drip irrigation	Without irrigation	Drip irrigation	Without irrigation	Drip irrigation	Without irrigation
Ilona	84.7	48.7	119.2	50.1	834.4	350.7	41,720	17,535
Futura	83.8	52.3	108.7	48.3	782,6	338.1	39,130	16,905
Dura	87.2	55.2	114.2	49.1	776.6	304.4	38,830	15,520
Rostara	86.8	53.4	106.5	47.5	734.8	308.7	36,740	15,437
Average	*85.6*	*52.4*	*112.2*	*48.8*	*782.1*	*325.5*	*39,105*	*16,349*

Conclusions

1. Drip irrigation is a method that could contribute to the extinction of the potato crop in South Romania, including on soils not so favorable to the potato crop, with high clay content respectively.
2. Drip irrigation assures good growth and development conditions for the potato plant which is positively correlated with the average weight of tuber, average yield per plant and per surface unit.
3. Under the climatic conditions of 2006 and on a soil with high content of clay, the drip irrigation determined compared with no irrigation for four potato varieties a plus of 63.3% for the height of plant, 129.9% for the average weight of tuber, 140,2% for the average yield per plant, and 139,1% for the average yield per hectare.

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MICROELEMENTS INFLUENCE ON POTATO YIELD FROM ALLUVIAL SOILS OF BRĂILA' S BIG ISLAND

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Key words: potato, microelements, genotype

INTRODUCTION

Potato has specific important physiological feature concerning macro and micro mineral nutrition which differ from another crops (Amberger, 1975, Borchmann et al.,1972, Cheng 1973, Boynd, Knesek, 1972, Stevenson, Ardakani, 1972).

Among microelements, Mo and Mn in interdependence with other technological sequences (*varieties, fertilization with N, P, K, application methods of microelements*) shows to be most efficient on yield and quality of potato crop from alluvial soils (Lindsay, 1972).

Research tackle with microelements, taking into account in this work, set out from the thought that on Brăila's Big Island ecosystem, could be met a high diversity on microelements level. An intensive agriculture which presume varieties with high quantitative and qualitative performance, exclusively chemical fertilization, with NPK and irrigation have therefore a decline of microelements level of these soils (Bajescu, Chiriac, 1984, Epstein, 1972, Gauch, 1972).

On alluvial soil from Brăila Big Island, microelements efficiency depends from several factors and genetic specificity is one of the most important.

There is a genotype specificity but not only, concerning ions absorption, transportation, distribution, accumulation on different vegetative organs, re-utilization, desorbtion and microelements part on growth and development processes. (Brown et al., 1972, Borlan et al., 1992, 1994, Mangel, Kirkby, 1992).

MATERIAL AND METHODS

The researchers were carried out between 1988-2007. The goal was to establish the microelements influence (Mn, Mo) in interaction with their application method (soil, tuber, vegetation), macro-elements fertilization (N, P, K) and genotype used on potato yield, productive and physiological potential with extremely importance on productive potential of this plant on Braila Big Island ecosystem.

The researchers were done on a poly-factorial layout with subdivided plots 3 R x 3A x 3B x 3C x 3D. Studied factors were: A- genotype (Impala, Kondor, Sante); B- NPK fertilization (260, 520, 780 kg a.i /ha); C- microelements (control, Mo, Mn); D- microelements application system (on soil, tubers, vegetation). It was used the irrigation system, which assured a limit of 75-80% from IUA on the hole vegetation period.

For statistical calculation was used MSTAT-C and SPSS program.

RESULTS

From experimental results we concluded that Mo and Mn application has positive effects on different potato yield elements of seed potato varieties. The varieties has been significant differentiated from total yield level on all varieties. The decreased order of varieties was: Kondor, Sante and Impala (Table 1.) After Mo application an average yield progress was achieved: 4.6-6.2 t/ha (6.9-11.3%). After Mn application the average yield progress of all three varieties was 6.7-8.8 t/ha (10.0-16.1%). After application of Mn and Mo the highest yield progress was on Sante variety.

Table 1. Microelements and varieties influence on potato yield Braila' s Big Island 1988-2007

Micro elements	Tuber yield					
	Impala		Kondor		Sante	
	t/ha	Cv %	t/ha	Cv %	t/ha	Cv %
Control	50.8 i	14.2	66.9 c	17.9	54.8 h	18.1
Mo	55.5 g	13.5	71.5 b	16.4	61.0 e	17.4
Mn	57.5 h	12.7	73.6 a	16.1	63.6 d	15.6

DL 5 % = 0.3 t/ha

After application of microelements, at all studied varieties a decreased on variant coefficients of average yield was noticed.

Through their characteristics concerning precocity, productivity, their nutritional necessities, after application with Mn and Mo, varieties reacts different toward total dray matter obtained on vegetation period.

Table 2. Microelements and varities influence towards total dry matter production Braila´s Big Island (1988-2007)

Micro elements	Dry matter production					
	Impala		Kondor		Sante	
	t/ha	Cv %	t/ha	Cv %	t/ha	Cv %
Control	14.2 i	16.5	19.2 e	30.2	15.9 h	19.4
Mo	17.6 g	14.2	23.3 b	23.1	20.2 d	21.9
Mn	18.7 f	14.0	24.5 a	19.5	21.4 c	18.4

DL 5 % = 0.1 t/he

Kondor and Sante varieties with productive high capacity, reacts more powerful on microelements application with yield progress over 4.1-4.3 d.m/ha comparative with control variant (Impala-3.4 t d.m/ha). Through Mn application, significant yield progress has obtained 5.3-5.5 t d.m/ha comparative with control variant (Impala 4.5 t d.m/ha). As well as in fresh yield, a decreased tendency of variation coefficient has been observed after Mo application and ever more pronounced after Mn application.

Through Mo application on all varieties the harvest index grows meaningful from about 0.67 to 0.70 and after Mn application to 0.72. The differences between varieties were insignificant.

Table 3. Microelements and variety influence on Harvest IndexBraila's Big Island (1988-2007)

Micro elements	Harvest Index					
	Impala		Kondor		Sante	
	Harvest Index	Cv %	Harvest Index	Cv %	Harvest Index	Cv %
Control	.675 c	4.1	.671 c	4.3	.668 c	7.9
Mo	.705 b	3.9	.698 b	4.0	.705 b	4.7
Mn	.718 a	3.3	.715 a	3.0	.715 a	3.8

DL 5 % = 008

After Mo and Mn application on these varieties with different precociousness, ecological and productivity potentiality, *Harvest Index* variation coefficient decreased (Table 3.)

Positive effect of Mo and Mn microelements on total potato yield become manifest on the hole level of soil NPK fertilization.

Table 4. The influence of microelements and complex fertilizer with NPK on potato yield Braila's Big Island (1988-2007)

Micro elements	Tubers yield/ ha					
	260 kg a.i NPK		520 kg a.i NPK		780 kg a.i NPK	
	t/ha	Cv %	t/ha	Cv %	t/ha	Cv %
Control	48.7 i	13.1	60.0 f	13.2	63.0e	19.2
Mo	52.0h	10.3	64.9d	12.8	71.1b	16.9
Mn	54.1g	10.1	67.2 c	10.9	73.3a	18.6

DL 5 % = 0.3 t / ha

NPK fertilization level determine significant reactions of total potato yield, on interaction with applied microelements (Mo and MN). Thus, the highest reaction, meaningfully statistical, is realized on 780 kg/ha a.i NPK level, after application of Mn (16.3%), and then followed by Mo (12.5%), on the same NPK level. Minimum level of NPK fertilization, about 260 kg/ka, realize the lowest yield increase, about 11.0 % with Mn and 10.6 % with Mo, meaningfully statistical. It comes out that in this interaction also, the Mn effect is superior to those make by Mo (Table 4.)

Table 5. The influence of microelements and complex fertilization with NPK on dray matter production Brăila's Big Island (1988-2007)

Micro elements	Dray matter production / ha					
	260 kg a.i NPK		520 kg a.i NPK		780 kg a.i NPK	
	t/ha	Cv %	t/ha	Cv %	t/ha	Cv %
Control	12.4 i	13.8	17.3 e	15.2	19.6 e	21.6
Mo	13.6 h	13.3	21.7 d	13.8	25.5 b	16.6
Mn	14.1 g	12.8	23.4 c	11.8	27.3 a	19.8

DL 5 % = 0.1 t/ha

NPK fertilization determine, significant changes of total dray biomass also, realized on special conditions of Braila's Big Island alluvial soils. Indifferent of fertilization level, Mn ascertain increase in total dray biomass between 13.7 %-39.2 %, meaningfully statistical. These increases due Mn, growths with NPK higher doses. On the same time, Mo, ascertain dray biomass growths with macroelements fertilization increases, till 30.1 % (Table 5.)

Table 6. The influence of microelements and complex fertilization with NPK on Harvest Index Brăila' s Big Island (1988-2007)

Micro elements	Harvest Index					
	260 kg a.i NPK		520 kg a.i NPK		780 kg a.i NPK	
	Harvest Index	Cv %	Harvest Index	Cv %	Harvest Index	Cv %
Control	.690 d	3.5	.688 e	4.3	.656 e	4.2
Mo	.699 c	3.4	.699c	3.8	.709 b	3.8
Mn	.709b	2.9	.714ab	2.8	.721a	4.2

DL 5 % = .008

Blending microelements Mn and Mo application with complex fertilization with NPK determine in this case also, significant changes of *Harvest Index*, productive structure of potato with a high technological and productive importance. The highest value of this index, meaningfully statistical, is obtain on maximum NPK fertilization level – 780 kg a.i/ha + Mn application. Average NPK level-520 kg a.i/ha, applied with Mn, ascertain an equal signification index with anterior variant of NPK fertilization (Table 6.)

CONCLUSIONS

- applications with Mn and Mo microelements ascertain yields increase and their constancy of irrigated potato cultivated on alluvial soils from Danube precinct dyke.
- on studied potato varieties, Mn applications has higher effects than Mo applications.

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CLIMATIC CHANGES AND POTATO CROP PRODUCTION IN THE CENTRAL PART OF ROMANIA

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Key words: Rainfalls, temperature, hydrothermal index, potato yield.

Summary

In the last period the potato in Romania is confronted with some problems linked to climatic and social changes (dramatic reduction of surfaces, diminish of resources, the quality of planting material). The paper presents a multiyear study regarding the climatic changes in Brasov area (center of Romania) and their implications in potato crop.

In the period 1961-2007 the annual temperature average grow from 7.3°C to 8.0°C and the sum of the annual rainfalls decrease from 620.0 mm to 570.0 mm.

For a period of around 100 years (1910-2007) it was calculated the variation and the tendency of hydrothermal index. It takes in consideration the rainfalls during the winter and the coefficient of soil retention, the rainfalls quantity and the temperatures sum (>0.0°C) in vegetation period. This index is correlated better with the performances of potato crop than the temperature and rainfalls.

A comparative study was done of the dynamics of the growth rate of yield accumulation for three potato varieties, OSTARA (early), ROCLAS (moderately early) and SANTE (moderately late), in the two year period, 1999 (normal) and 2000 (very dry) relief as follows:

Introducton

Potato is one of the most important crop plant, presenting a large ecological plasticity, and being cultivated worldwide, in over 140 countries.

Identification of the response to the applied culture technology of the cultivated potato plants in the specific growth conditions, has a great importance in describing and measuring of processes used for model and stimulation of plant growth with the regard of the yield accumulation.

Methods and Material

The biological material was represented by three regional varieties OSTARA (early), ROCLAS (moderately early) and SANTE (moderately late). These varieties were studied in different growing climatic conditions, realized by planting the plant material in various planting times, in years 1999 (a normal year from climatic point of view) and 2000 (an extremely dried year), at least for the Brasov area (central part of Romania, figure 1).

Pluviometric and thermal characterization was made for 52 years (1961-2007).

Hydrothermal index was calculated: $K = (0.6H+Q)/0.1\Sigma t^0$

K = hydrothermal index

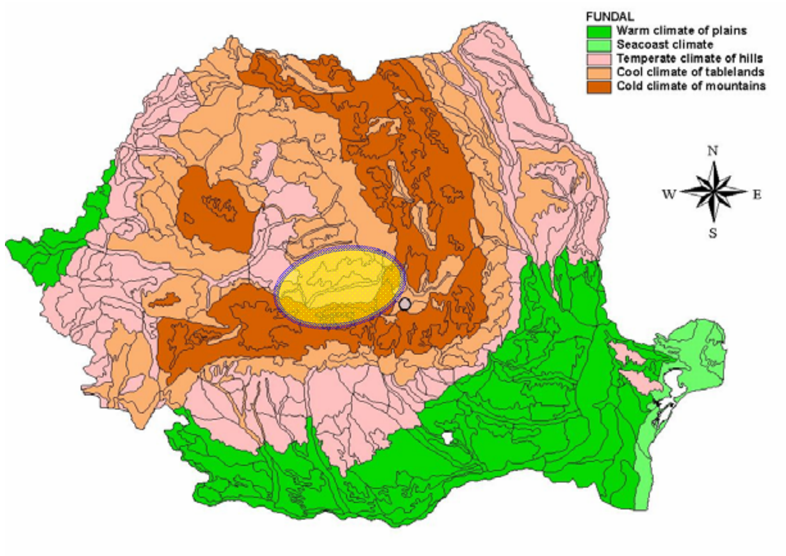
H = rainfalls sum in the course of November – March

0.6 = coefficient to store in sol the water come from rainfalls in November – March period

Q = rainfalls amount in respective season (K1….K4)

$\Sigma t°$ = temperature sum >0° from respective season.

Figure 1. Climatic zone of Romania



Results

CLIMATE

In the period 1961-2007 the annual temperature average grow from 7.3°C to 8.0°C (figure 2) and the sum of the annual rainfalls decrease from 620.0 mm to 570.0 mm (figure 3).

The variation of hydrothermal index between 2 and 3 represents an optimum for potato crop (it isn't required the irrigation). In Brasov area, in the studied interval, the hydrothermal index decrease constantly from 2.55 to 1.25 (figure 4), in the last 15-20 years being necessary a water supplementary by irrigation for a profitable crop potato.

Figure 2. Average of annual temperature (1961-2007)

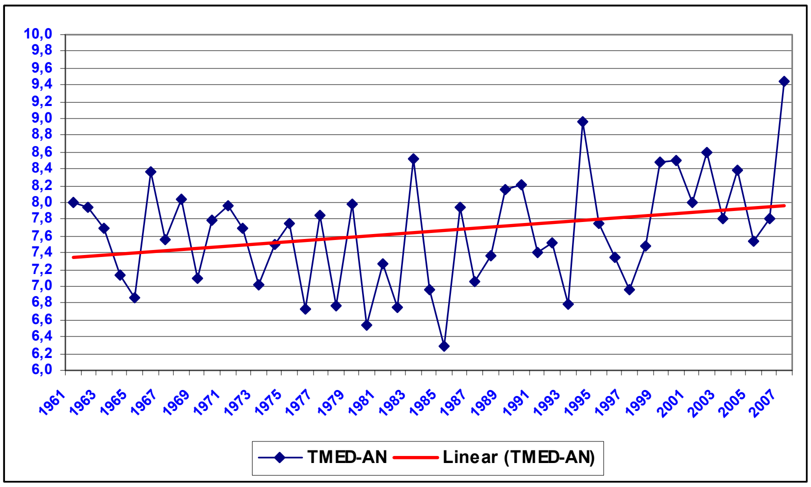
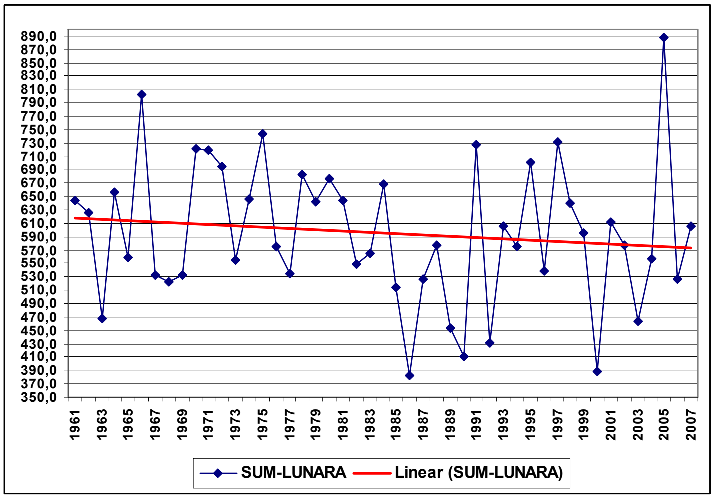
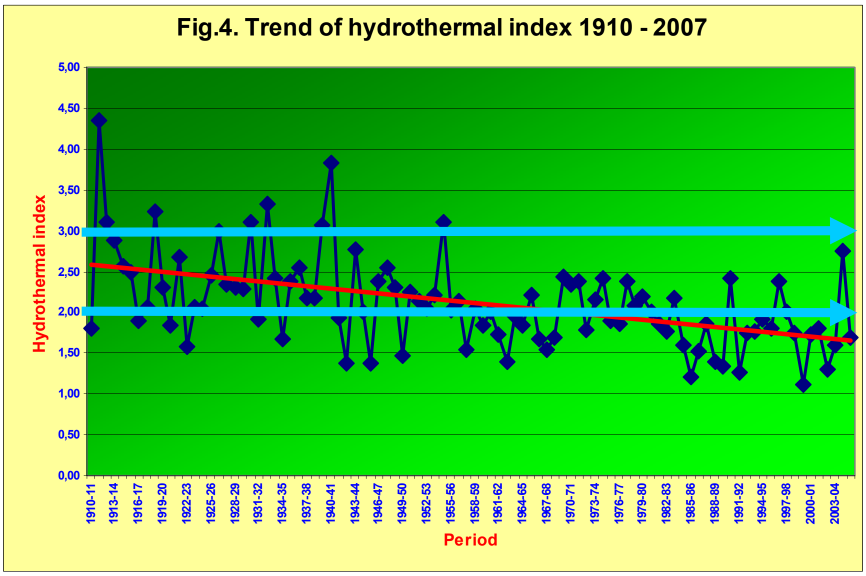


Figure 3. Sum of annual rainfalls (1961-2007)





YIELD

All varieties were affected by the drought of the 2000 year (figure 5, 6 and 7).

The yields varied in 1999 from 35 t/ha (OSTARA) to 50 t/ha (SANTE).

In year 2000, the productions varied between 6.5 t/ha at the OSTARA variety and 23 t/ha at the ROCLAS variety.

Compared with the year 1999, in the drought conditions of the year 2000, the ROCLAS variety had the best performance, presenting a decreasing value of 54% of the yield, when compared with the OSTARA variety, which had the lowest rate of production (81%).

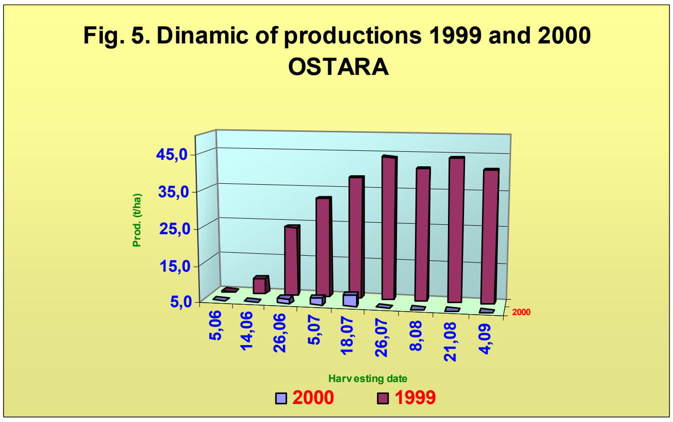


Fig. 5. Dinamic of productions 1999 and 2000
OSTARA
45,0
35,0
25,0
15,0
5,0
Prod. (t/ha)
5,06
14,06
26,06
5,07
18,07
26,07
8,08
21,08
4,09
2000
Harvesting date
2000
1999

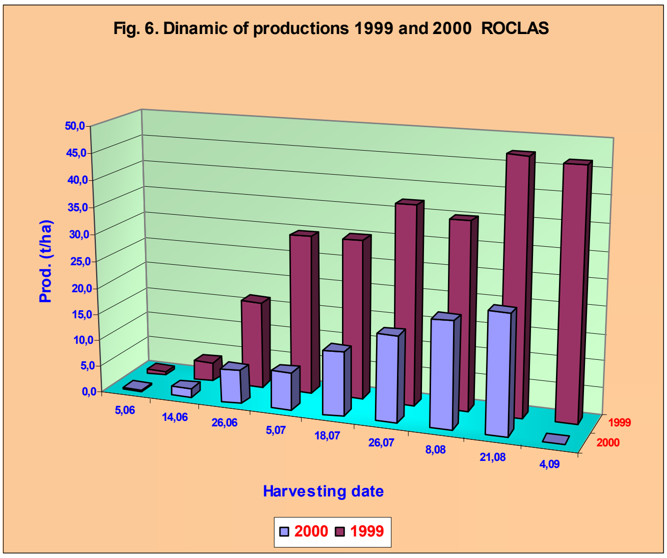


Fig. 6. Dinamic of productions 1999 and 2000 ROCLAS
50,0
45,0
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
Prod. (t/ha)
5,06
14,06
26,06
5,07
18,07
26,07
8,08
21,08
4,09
1999
2000
Harvesting date
2000
1999

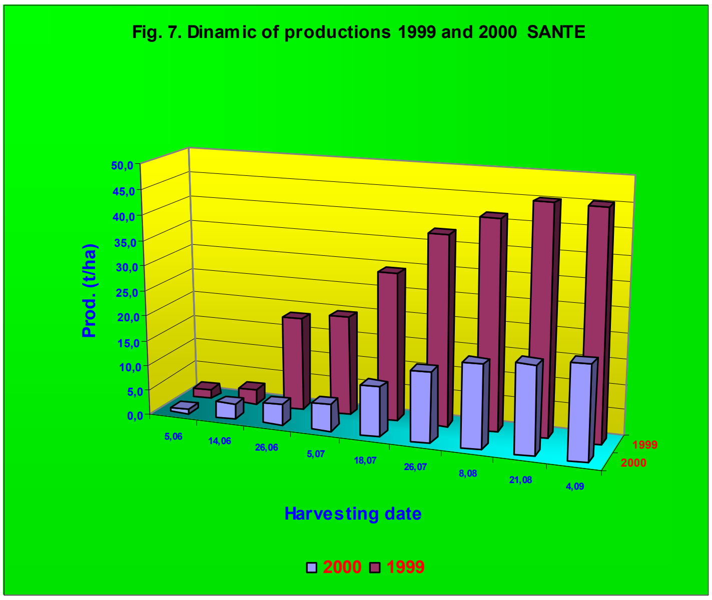


Fig. 7. Dinamic of productions 1999 and 2000 SANTE
50,0
45,0
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
Prod. (t/ha)
5,06
14,06
26,06
5,07
18,07
26,07
8,08
21,08
4,09
1999
2000
Harvesting date
2000
1999

PRECISION AND ORGANIC FARMING

WEED MAPPING – AN IMPORTANT PART OF THE PRECISION FARMING

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Three-year field trials performed during 2005 – 2007 were focused on the study of weed distribution on selected plots with growing of potatoes. In the period of potato emergence, coordinates were determined for each point in the grid of 20x20 m using of GPS kit GPSMap 276. and values of distribution of all present weed species (g green mass/m^2) were assigned to the coordinates. Results were derived of critical amount of present weed species per area unit in relation to yield reduction. Based on these findings and maps of weed distribution on a concrete field plot data for map construction of treatments (spraying) were designed.

Key words: precision farming, potato, weeds, GPS, mapping, herbicides

Introduction

In intensive agricultural production application of pesticides is inevitable. Within the rules of sustainable agriculture and good agricultural practice, target applications of pesticides are necessary. There are many mechanisms, how to make pesticide applications more effective and how to reduce application rates. However, herbicide applications are overall done, often without actual knowledge of weed infestation intensity and occurrence of individual weed species. Consumption of herbicides is at least 50 percent and more from the consumption of all pesticides.

Development of application techniques, possibility of GPS signal use for navigation and rapid advance in electronics opened a way for local pesticide application in dependence on concrete conditions.

The philosophy of precision farming is the individual management of field segments, not field as a whole. The precision farming is currently used especially in fertilizer application and cereal growing technology. It depends on availability of required machinery and the whole technologies based on this principle. In the research field another elements are verified that are in accordance with principles of precision farming. Weed management is one of them.

Herbicides constitute the largest part of pesticide inputs into agriculture at present. In this area noticeable savings could be provided. A series of scientific studies (Werner et Garde, 1998, Clay et al., 1999, Nordmeyer et Häusler, 2000, ref. Balík, 2005) indicate that occurrence of weeds is very uneven also within one plot. Local specific weed management based on principle of precision farming supposes that in sites, where weed occurrence is absent or below the threshold, application of product will be not done and in treated parts the rate will be adjusted according to degree of weed infestation. However, use of principle of local specific weed management supposes that weed infestation of the plot is mapped on sufficiently detailed level. Creating information about distribution of individual species and their aggregation it is necessary to near as much as possible to the reality and simultaneously to keep time consumption on low level. Most frequently, the mapping has been done with direct evaluation of the crop, but it is time consuming. Several literature resources (Krohman et al., 2002, Werner et Garde, 1998, ref. Balík, 2005) indicate that time consumption could be partly reduced by using of maps from previous years, since range of species and weed frequency does not markedly differ due to certain site stability within one year. In recent years, it is also possible to record efforts in using of sensors for automatic weed detection.

To apply precision farming in the technology of potato growing is so far difficult. Application maps based on soil analyses, soil characteristics or pest and disease occurrence are not comparable to yielding maps in a higher degree. The reasons are technical problems associated with obtaining the data for creation of yielding maps. The harvester of cereals has a mounted flow sensor; in potatoes it is difficult to record a definite yield in certain time, on a certain place. It is due to the conditions of potato harvest (surface non-roughness, great shocks) and amounts of admixtures. Systems based on optical scanning using CCD camera (in addition to weight, tuber size and shape is recorded) and a mechanical system with a bounce plate and a loading cell (Molema and Hofstee, 2002, Ehlert, 2002) have been developed.

The principles of precision farming could be applied in potatoes regardless of the existence of yielding maps. It particularly concerns plant protection products, especially herbicides. Mapping the weed occurrence in the potato crops is considered an important priority in the introduction of new growing technologies (http://northeastipm.org/priority/potatoesNY_2003.html).

Weeds are very important harmful agents in potato growing. Depending on species range and intensity of the occurrence they could cause a reduction of tuber yields over 60 %. The weeds compete with potato plants in relation to all growing and developmental conditions. They shade young potato plants and deprive them of sunshine, have requirements for soil moisture and nutrients, they are able to take more moisture from the soil compared to potatoes, therefore they grow more rapidly and prevail over potatoes, they possess better absorption capability and enhance risk of mechanical tuber damage at the harvest.

Methods

The aim of this study was to find, whether spatial variability of individual weed species exists within the technology of potato growing in the Czech Republic, in that it would be possible to perform local herbicide application using of a suitable technique.

The study was done between 2004 and 2007 in agricultural enterprises with long-term specialization in potato growing, i.e. having potato area larger than 150 ha (10 % concentration of potatoes in crop rotation).

Weed samplings were done based on a systematic scheme, when a field was divided into regular grid of points constituting squares of 20 x 20 m. In total, 179 points were created in the systematic scheme in 2004, 109 points were created on one workplace and 206 on other workplace in 2005, 62 points in 2006 and 41 points were created in 2007.

In each point species range and intensity of weed distribution were determined on the area of 1 m^2. Twenty-six weed species were studied (number of individual plants from each species in pieces. m^2, weight of weeds from each weed species in $g.m^{-2}$).

Sampling date was 35-40 days after planting. An example of mean occurrence of selected species over the whole field is given in Tab. 1.

For each point coordinates of northern latitude (x) and eastern longitude (y) were determined in global coordinate system UTM (Universal Transverse Mercator) and WGS84 using of a GPS kit GPSMap 276. GPS data were primarily processed in software MapSource. Maps of weed distribution were constructed after setting the values of individual weed species (z) using of mapping software Surfer 8.04 (data interpolation was performed by krigging approach).

Tab. 1: Example of mean values of weed occurrence in pieces . m^{-2}

Weed/studied year	2004	2005	2006	2007
Agropyron repens	23,1	5,86	0,03	0
Cirsium arvense	4,4	0,28	0	0
Sonchus arvensis	1,1	0,50	0,80	0
Viola arvensis	212,8	1,00	40,46	28,46
Fagopyrum convolvulus	9,2	77,77	30,68	0,35
Polygonum persicaria	0,6	36,18	4,68	0,10

Results

Mean values do not reflect variability of occurrence of dangerous weed species. It could be partially expressed by histograms, indicating individual and cumulative frequency of individual species in sampling grid according to the systematic scheme.

Effective expression of spatial variability of occurrence could be only provided by mapping using of GPS and appropriate software.

For each plot, maps of all occurring weeds were constructed in individual years of study. It was found that degree of variability of occurrence is dependent on weed species. For fulfilment of set target, i.e. use of local herbicide application, division of weeds into three groups was designed:

a) a group of dicotyledonous weeds that are in regular distributed with lower variability on a plot and selection of broadcast or local application is dependent on total intensity of the occurrence. The examples are given in Figs. 4

b) a group of monocotyledonous weeds represented by Agropyron repens and Echinochloa crus-galli, which occur on a plot with higher variability and a possibility of local application is higher. The examples are given in Figs.5

c) a group of dicotyledonous weeds, which are represented by Sonchus arvensis and Cirsium arvense with very high variability of their occurrence on a plot (often also after first overall treatment with a herbicide) and local application can be done almost every time. The examples are given in Figs. 6

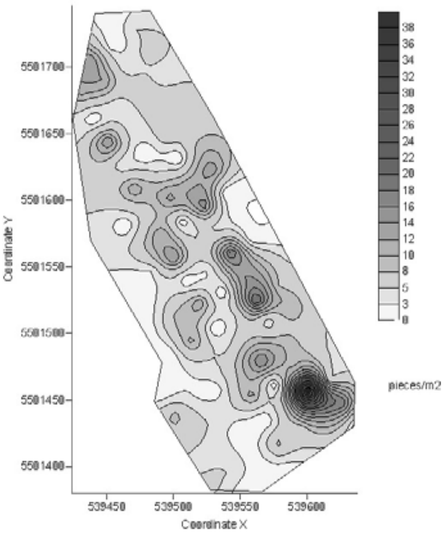

Fig. 4: Map of Viola arvensis occurrence (2006)

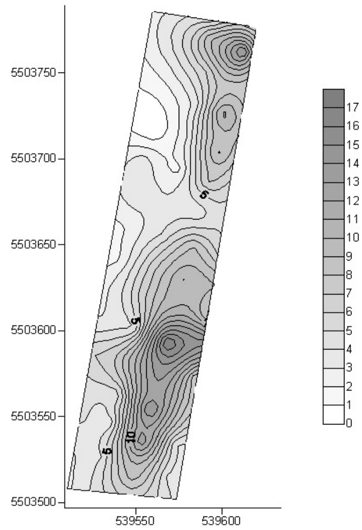

Fig. 5: Map of Agropyron repens occurrence (2005)

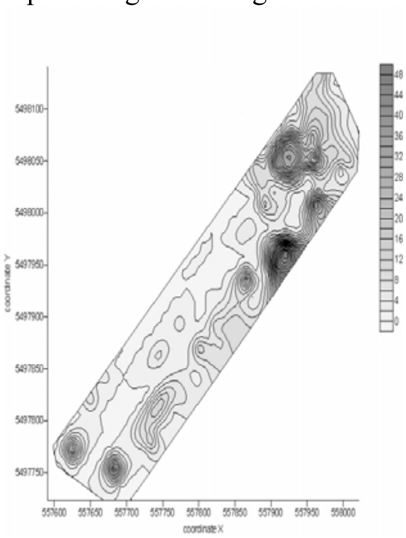

Fig. 6: Map of Sonchus arvensis ccurrence (2005)

The efficiency of a treatment is decided by occurrence of a weed in comparison to threshold critical amount. Critical amount is such a value of occurrence per area unit, in which cultivated crop is endangered due to weed competition ability. We determined critical amounts of weeds in parallel established trials. Hitherto results indicate that e.g. five plants per 1 m^2 are critical amount in Agropyron repens, 2 plants in Galim aparine, Sonchus arvensis and Chenopodium album and only 1 plant in volunteer rape and Cirsium arvense.

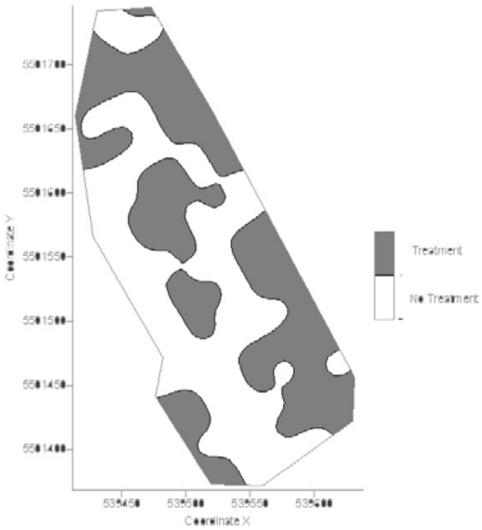

Fig. 7: Application map of Olešná 2005

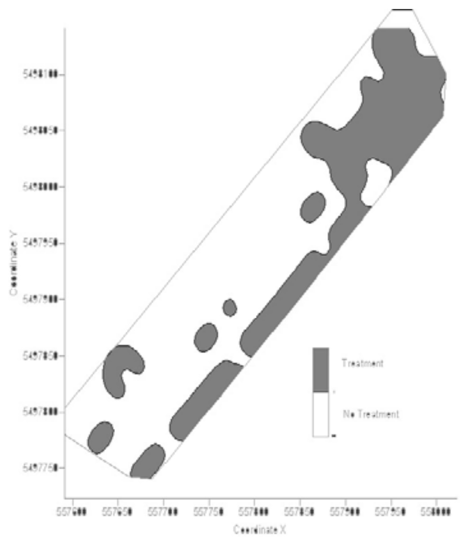

Fig. 8: Application map of Borová 2005

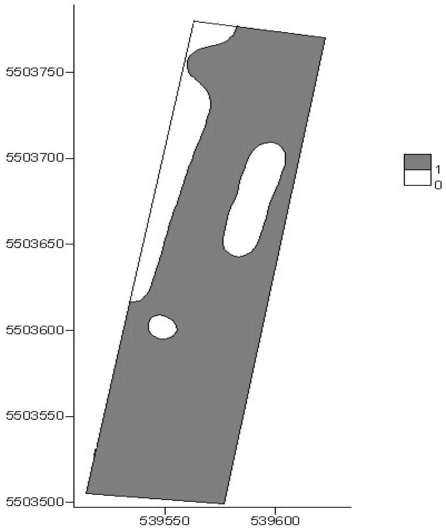

Fig. 9: Application map of Olešná 2006

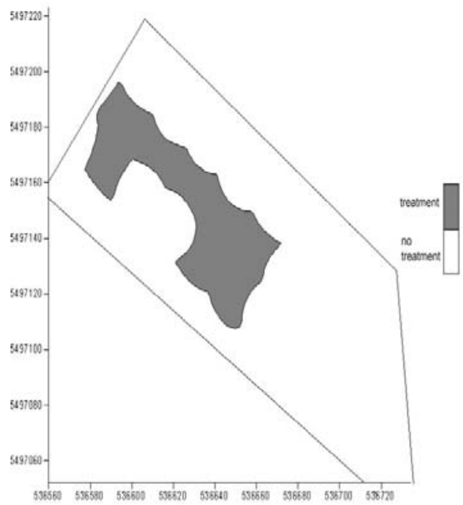

Fig.10: Application map of Okrouhlice 2007

This critical number is taken away from concrete value of the occurrence in a studied point. If the sum of all results in all weeds is more than 1, the value YES (1) is assigned to the point, if the sum is nil, value NO (0) is assigned to the point.

Fig. 7 illustrates an application map from the year 2005 in the enterprise Olešná, where 47 % of herbicide could be saved, Fig. 8 presents an application map from the enterprise Borová with a saving of 57 % of herbicide. On contrary, saving in the enterprise Olešná would not be substantial in the year 2006 (Fig. 9. In 2007 44 % of herbicide could be saved according to an application map in the enterprise Okrouhlice (Fig. 10).

Conclusions

The results confirmed the possibility of obtaining information about spatial variability of the weeds according to species using of GPS and mapping software and to respect this variability in management of mono- or dicotyledonous weeds. Cost will be saved and pesticide-loading of environment will be reduced.

Acknowledgements

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New York State Potato Industry Priorities

http://northeastipm.org/priority/potatoesNY_2003.html

THE CONTROL OF STEM BLIGHT AND THE SPREAD OF POTATO LATE BLIGHT BY COPPER SEED TREATMENT

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Key words: *Phytophthora infestans*, stem blight, Öko-Simphyt

Abstract

Late blight still is an unsolved problem in organic potato farming. Up to now the most effective way to control this disease is the use of copper fungicides. Transferring results from regular farming, seed treatments with copper fungicides shall postpone the beginning of the blight epidemic and decrease the spread of the disease with soil water. Thus stem blight, which can't be prevented otherwise, shall be reduced. In field trials, conducted 2005-2007, copper fungicide treatments reduced primary stem infections (stem blight) and the spreading of the pathogen from infected seed tubers.

Introduction

Potato late blight caused by the oomycete *Phytophthora infestans* is one of the most important yield-limiting factors in organic potato farming. The most effective way to control this pathogen is by the protective foliar application of copper fungicides. This can prevent secondary infections on the leaves, which are caused by wind-spread sporangia. Sources for this inoculum are volunteers and/or infected cull piles (HOFFMANN & SCHMUTTERER, 1999), especially after a mild winter. However latent infected tubers in storage resemble the main inoculum source (Andrivon, 1997). During storage these tubers don't show any symptoms, and are used as seed tubers, bringing the inoculum direct into the field (Adler, 2000). At high soil moisture after rainfall (20mm) the pathogen expands it's growth into the stem, causing stem blight (Zellner, 2006). This happens either by direct intercellular growth from the tuber into the stem (Adler, 2000; Appel et al., 2001), or by reinfection of the stem after sporulation on the surface of the tuber. Thus under suitable conditions early stem blight occurs, which can not be prevented by foliar application of fungicides. The produced sporangia can be spread via soil water to neighbouring plants (Bain & Möller, 1998), causing stem infections on them as well. It has been showed in regular farming, that the use of fungicide seed treatments can postpone primary infections for 8-20 day and slow down the course of the blight epidemic (Bäßler et al., 2002). These findings shall be transferred as a new approach into organic farming within the project Öko-Simphyt. Copper seed treatment shall prevent healthy tubers from being infected by inoculum in the soil and also inhibit latent infected tubers directly from producing sporangia (Benker et al., 2006).

Materials and methods

In the years 2005 –2007 field trials where conducted at two sites in Bavaria on heavy and light soil. Seed tubers were artificially inoculated by injection of zoospores of *P. infestans* to produce latent infected tubers, which where treated with copper fungicides (Copperhydroxide, trade name: Cuprozin flüssig; 120g Cu/ha) before planting.

Two seed tubers were planted closely adjacent to each other. One of them (variety Quarta) was inoculated with 200 zoospores and the other one (variety Agria) remained healthy. Copper seed treatment was either applied to the infected tuber or the healthy one. For control both tubers remained untreated. The frequency of stem blight was measured weekly and confirmed by PCR detection (JUDELSON & TOOLEY, 2000). In this way the effect of the copper seed treatment on the spreading of inoculum from diseased to healthy plants was tested.

Results

If both tubers where not treated with copper, the latent infected Quarta was able to infect the neighbouring plant, causing stem blight at a frequency of 45% (figure 1). A copper seed treatment on the infected Quarta stopped the pathogen from spreading to a certain degree, reducing the frequency of stem infections on the adjacent Agria for 10%. The best result was achieved by a copper seed treatment of the healthy Agria, significantly preventing the infected Quarta from infecting the neighbouring plants by released sporangia (25% frequency of stem blight).

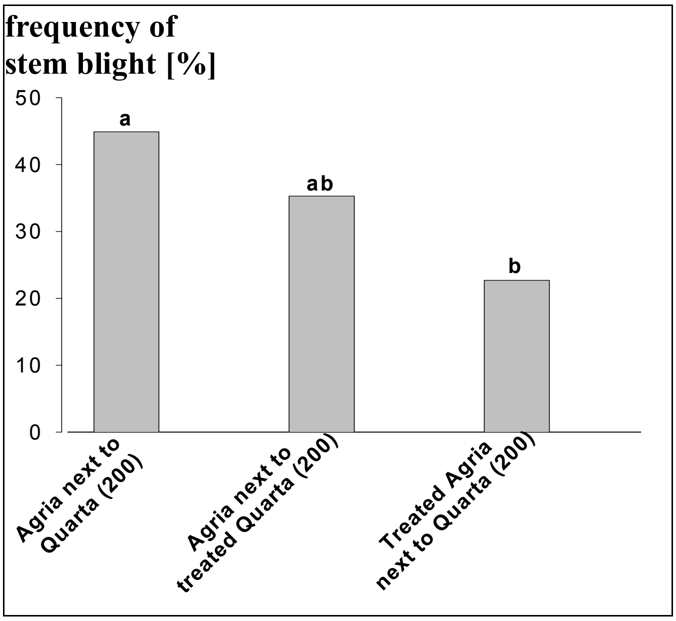

Figure 1: Effect of copper seed treatment (120g Cu/ha) on stem blight; different letters indicate significant ($p<0.05$) differences; variety Quarta was artificially inoculated with 200 zoospores.

Throughout the 3 years data from 56 experiments with copper seed treatments was collected. Figure 2 shows the overall effect. In 47% of the experiments a reduction of stem infections of less than 10% was achieved. The effectiveness of a copper seed treatment was 10-50% in 35% of the experiments. A high effectiveness above 50% was achieved in 18% of all experiments.

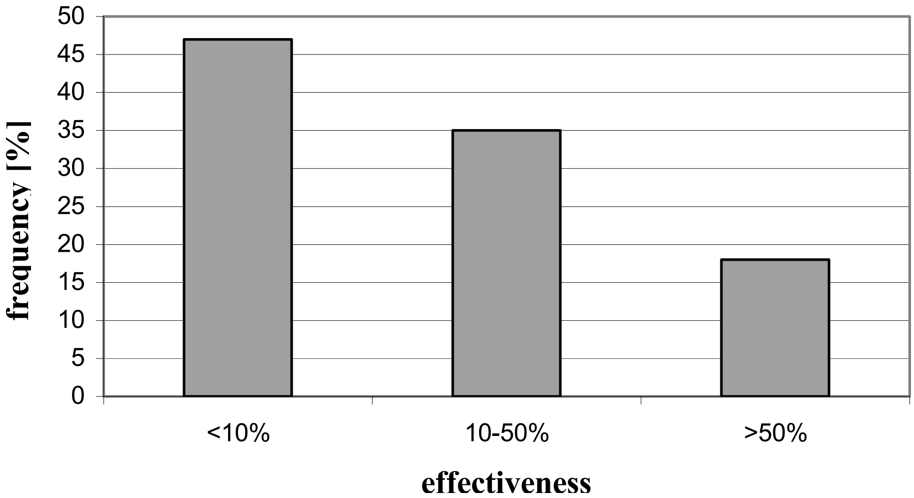

Figure 2: Effectiveness of a copper seed treatment against stem blight in 56 experiments.

Discussion

Other than secondary infections, stem blight (primary infections) can not be prevented by the protective foliar application of copper fungicides. And since the source of inoculum is brought into the field with latent infected seed tubers, crop rotation doesn't work. Up to now the only way to prevent stem blight and the spread of the pathogen caused by infected seed tubers is the usage of copper seed treatment. The applied dosage of the copper is as low as 48g/t (120g/ha), enabling also foliar applications of copper against secondary infections to the maximum allowed amount of copper (3000g/ha per year in Germany).

Conclusions

Copper seed treatments are a promising way to reduce stem blight and decrease the spread of inoculum to neighbouring plants. This new method of applying copper can prevent the early outbreak and fast course of the epidemic.

Acknowledgments

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POTENTIALS FOR WIREWORM CONTROL IN ORGANIC FARMING

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Ware potato production plays an important role for the profitability of many organic farms. In 2006 app. 6.700 ha of ware potatoes were produced in Germany according to the EU - regulation 2092/91 on Organic Farming (Engelhardt & Schaack 2007). High tuber yield and quality are the main objectives of organic potato production. Many pests and diseases not only affect yield but also tuber quality. Damages caused by wireworms can result in significant economic losses to organic potato growers (Keiser et al. 2005) and are difficult to control. During the last years we have investigated agronomic approaches to limit damages, which were completed by basic research on the reproduction behaviour of the beetles using pheromones. Organic potato field trials were carried out in Southern North Rhine-Westphalia, targeted on assessing the impact of variety choice and harvest date on wireworm damage of potato tubers. Tuber yield and quality were recorded and submitted to ANOVA. Variety choice had a significant effect on tuber losses caused by wireworms. The amount of wireworm holes in cv. Princess was significantly higher compared with cv. Nicola. According to our findings the different sensitivity to wireworm damage was not linked to early tuber development and the reasons for these findings yet remain unknown. Bringing forward tuber lifting dates to the middle of August resulted in significantly lower wireworm induced tuber losses compared with middle of September (Neuhoff et al. 2007).

Field assessments on the experimental farm 'Wiesengut' close to Bonn, Germany using pheromone traps confirmed that the main click beetle species in Germany are *Agriotes lineatus* and *A. obscurus (* Furlan & Toth 1999). Experiments focussed on estimating the range of attractiveness of the pheromone traps indicated that the recovery rate of the released beetles (*A. lineatus* and *A. obscurus*) to the traps was mainly affected by release distance, but also by time. Comparatively high recovery rates for both species > 60% were recorded for release distances up to 10 m in clover grass, while less than 10% of the beetles released at a distance of 60 m returned to the trap. More than 50% of the males were recovered within the first 24 hours (Sufyan et al. 2007). In a so called prevention trial male click beetles have been captured by pheromone traps and continually removed since three years (2004 - 2007). Wireworm abundance and development over time has continually been monitored in comparison with untreated control plots (no male trapping). While at some assessment dates a tendency to decreased wireworm population was noted in plots with the permanent presence of sex pheromone traps, recent assessments in autumn 2007 do not support the hypothesis of a cleaning effect induced by male trapping. The experiments suggest that a relatively high number of traps or an extension of the trapping periods have to be considered to reduce wireworm population in the soil via mass trapping. Whether or not male trapping can help to reduce wireworm damages in potatoes still remains unclear. Furthermore it is still unknown in how far the monitoring of male beetles flight can serve to predict wireworm damages in agricultural crops. For that purpose more basic research on the biology of wireworms is needed. Future control strategies for wireworms will have to be based predominantly on agronomic approaches.

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YIELD AND QUALITY DIFFERENCES A FEW POTATO CULTIVARS GROWING IN ORGANIC AND INTEGRATED CROP PRODUCTION SYSTEMS

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SUMMARY

In the years 2005-2007 an experiment aimed to comparison a few potato cultivars in two crop production system: organic and integrated was carried out. Eight cultivars from different earliness and different resistance to Phytophtora infestsns were assessed. The significant differences as well as yield and some tubers characteristics and chemical components were found. In organic system an average yield was about 30 % lower than in integrated one. In this system more tubers damaged by worms and more internal disorders were found. There were significant differences in chemical components of tuber. In organic system higher content of dry matter, and starch was obtained. There were no differences in vitamin C and nitrate content between production systems. Significant differences were proved only between cultivars.

INTRODUCTION

Potato is still one of the most important crop in Poland although in last years the area of this plant continuously decreases**.** Most of potato production comes from conventional system. There is still shortage of potatoes growing in organic and integrated systems and demands of people for this kind of potatoes getting increase. Potato is difficult crop specially for organic system because of high menace of agrofages, mainly Colorado beetle and Phytophtora infestans. The important thing is also cultivar selection. High resistance to late blight, good yielding and sufficient tuber quality is necessary to get success. Most experiments connected with potato organic production aim to assess cultivars for this system. The aim of this study was comparison 8 potato cultivars growing in organic and integrated system according to yielding, tuber quality and chemical components. These results can be useful for recommendation cultivars for 2 mentioned above crop production systems.

MATERIALS AND METHODS

The experiment was carried out in the years 2005-2007 on medium soil in two crop production systems: organic and integrated. In each system different crop rotation and different technologies were used.

Crop rotation in organic system : potato > spring barley with red clover > red clover with grasses (2 years) > winter wheat + catch crop

Crop rotation in integrated system : potato > spring barley > faba bean > winter wheat + catch crop.

In organic system only natural manure was used. For Colorado beetle control – bacterial insecticide Novodor was applied and against late blight cooper fungicides was used. Weed control was only mechanical. In integrated system the organic manure, mineral N- (80 kg per ha) and P and K to balance its intake in rotation was used. Chemical control was used only after exceeding harmfulness threshold of agrophages. In weed control mechanical – chemical methods were used. 8 table cultivars of different earliness from very early to late were tested. The cultivars were chosen according to the highest resistant to Phytophtora.

After harvest such elements were assessed: total and marketable yield, external quality of tubers (infection by Common scab, damages by worms, tuber deformations), internal (rust spot, hallow heart). Chemical components: dry matter, starch, vitamin C and nitrates contents were assessed.

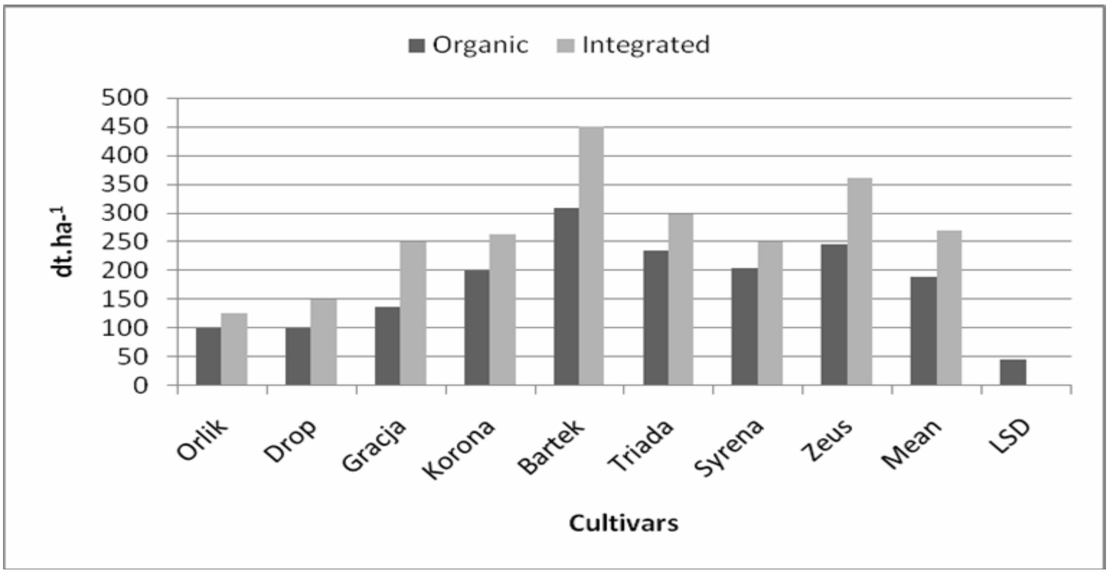
Table 1. Characteristics of tested cultivars.

Cultivar	Earliness	Late blight resistance (foliage)
Orlik	Very early	3
Drop	Very early	3
Gracja	Early	3
Korona	Early	3
Bartek	Middle early	5
Triada	Middle early	4
Syrena	Middle late	5
Zeus	Middle late	6

RESULTS AND DISSCUTION

There were significant differences in yielding between 2 production systems. An average yield for organic system was 188 $dt.ha^{-1}$ and 268 $dt.ha^{-1}$ for integrated one. Significant differences were observed between cultivars too. The lowest yield was obtained (for both production systems) for early cultivars. The highest level of yielding was achieved for middle early cultivar Bartek and middle late cultivar Zeus.

Fig.1. Yield of different cultivars in organic and integrated systems (average for 3 years).



2. Differences in yielding depending on vegetation periods.

The climatic conditions during vegetation period significantly influenced the yield in both systems but bigger differences were observed in organic system. The lowest yield in this system was noticed in 2005 (shortage of rainfalls and very bad their distribution) – the best in 2006. In the integrated system opposite situation had place – the lowest yields were in 2006 but the highest in 2007.

3. Differences in tuber quality.

There were not significant differences between production systems in such tuber characteristics as: infection by Cammon scab (there were only cultivar differences), share of green tubers . Significantly more tuber damaged by worms, and more internal disorders were found in organic system.

4. Differences in chemical components.

Significant differences between production systems in dry matter and starch content were found out . The quantity of vitamin C was higher in organic system, but it was not significant difference. Quite difficult interpretation considers nitrate content. There were no differences between 2 systems. It is almost obvious that in organic system (no mineral N) the level of this component should be lower. In this case such conditions as: good soil, rotation rich in papilionaceous plants, lower yield, and shortage of rainfall caused high level of nitrates in tubers came from organic system. Cultivars and years differences were also proved. The highest levels of nitrates were found in early cultivars in both systems.

Table 1.Differeces in tubers chemical composition depending on crop production system.

Crop production system	Dry matter content (%)	Starch content (%)	Vitamin C content mg%	Nitrate content $NaNO_3$ mg /kg fresh matter
Organic	22,00	14,82	20,07	245,4
Integrated	21,13	14,10	19,44	248,2
LSD	0,67	0,70	-	-

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BREEDING FOR ORGANIC FARMING: COMPARISON OF POTATO CLONES TRAITS ASSESMENT IN ORGANIC AND CONVENTIONAL CONDITIONS

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Introduction

The evaluation of potato varieties in organic fields brought out most important traits for organic potato production, which should contain organic potato variety. As the most important trait resistance to different pathogens, (late blight, black scurf, virus diseases, rhizoctonia etc.) has been notified (Zimnoch-Guzovska, 2003; Tiemens-Hulscher et al., 2003; Vogt-Kaute, 2001). The desired traits for organic potato breeding are adaptability to organic fertilizations (adequate root system, rapid juvenal root and plant development, good growth vigour, efficient mineral uptake and use), , ability to give yield in short growing period (early bulking and ripening, yield stability, reaching acceptable quality, good storability) (Tiemens-Hulscher et al., 2003) and compliance to marketing. A part of named traits are included in conventional potato breeding programmes, but some of characters are particularly significant for organic growing conditions. As conventional and organic growing conditions are different, requirements to varieties are different too; should these differences be a reason for creating separate breeding activities (Colon et al., 2003). Breeding for organic agriculture could took advantage that the expression of many traits is highly correlated between conventional agriculture and organic agriculture (Loschenberger, 2007). One of ways is to start breeding for organic farming in conventional programme, using so called indirect selection, and at defined generation evaluates probably acceptable clones in organic conditions. Because of the expected large plant – environment – management interactions under organic conditions the most efficient way is to start selection in organic field as early in the selection process as possible (Lammerts van Bueren, 2002). The selection in organic environment will include emphasis on rapid establishment, good ground cover, early bulking yield potential and tolerance to changeable humidity and fertility conditions through better root system (Bradshaw, 2007).

Materials and Methods

Nine potato clones (4th and 5th field generation) have been evaluated in conventional and organic fields for two years - 2006 and 2007. The clones were selected from existing potato breeding programme according assessment of leaf coverage, foliage resistance to late blight and maturity under conventional growing conditions. The variety 'Brasla' (medium late) was used as standard variety. The potato clones evaluation was done during growing period and after harvesting. The leaves resistance to pests was assessed visually (% of damaged leaves area) during disease development in the field. The length of growing period was determined counting days from emergence (80 % of planted tubers have sprouted) to end of vegetation. After harvesting yield and tuber size distribution were detected. The starch content in tubers was detected indirectly via specific gravity as percentage of fresh weight. The boiled tuber taste was assessed by expert panel using 9 point scale (9 – very tasty, 1- very nasty). The results of traits assessment in both environments' were compared. The main stress was on selection clones suitable to organic growing conditions.

Results

The foliage resistance to early blight was assessed in 2006 as late blight did not appear in the field due to extremely dry and relatively hot weather conditions. There was not found significant correlation between assessment in both growing environments ($r = 0.21 < r_{0.05,10} = 0.63$). Some other circumstates – ability of nutrition uptake, resistance to drought, foliage cover and others could affect clones resistance or sensitivity do early blight.

The distribution of late blight on potato clones foliage was assessed only in 2007. The spraying of fungicide was used in conventional potato field. The amount of damages in conventional field was less than in organic where spraying was not used. Correlation between late blight damage assessments in both environments was not significant ($r = 0.42 < r_{0.05,10} = 0.63$). It means, that genotypes assessment in conventional field with fungicide usage does not tell about genotypes resistance to late blight in organic conditions without fungicide usage. The evaluation to disease resistance has to be done in organic field for proper opinion.

The growing period was shorter in both environments in 2006 than 2007, because of extreme dry and quite hot weather, which fastened plant development and limited nutrient availability. The potato plants emerged 2 -3 days earlier in organic field than conventional both years due to seed tuber presprouting. But growing period for clones in organic field was shorter for about ten days than in conventional field (Table 1). One reason is that presprouting fastened whole plant development. The next reason, especially in 2007, was that late blight damaged foliage and interrupted vegetation in organic field earlier than in conventional. The growing period length differed between genotypes according predicted maturity time. There was not found significant relationship between assessments in organic field both years, but correlation between assessments in conventional field both years was significant. The growing period in organic environment was more affected by weather conditions and late blight distribution as in conventional environment.

Table 1. The traits of potato clones in organic and conventional fields in 2006 and 2007

Traits	Organic field				Conventional field			
	2006		2007		2006		2007	
	min-max	average	min-max	average	min-max	average	min-max	average
Growing peroid, days from emergence to the end of vegetation	57-71	63.7	54-90	74.8	65-83	77	80-102	87.9
Tuber yield, t ha^{-1}	10.1-28.4	16.1	17.0-35.1	24.6	4.7-41.8	23.1	33.7-65.0	44.8
Tubers > 50mm in yield, %	2-35	20	4-64	41	9-43	25	32-78	62
Starch content, %	13.9-21.4	17.2	14.9-19.4	16.2	11.5-19.1	15.0	12.2-19.3	15.6
Boiled tuber taste, points	6-7.5	6.8	6-7.8	7.1	6.3-7.7	7.0	6.1-7.8	6.9

The potato tuber yield was about twice lower in both environments in 2006 than in 2007 (Table 1). The influence of different weather conditions, affecting soil moisture was observed during trial. Tuber yield in organic growing conditions was lower than in conventional growing conditions both years. Correlation between yield assessments in both environments was significant in both trial years (Figure 2.). Significant relationship was found between tuber yields both years in the same environment. The selection of potential high yielded potato clones for organic growing conditions could be done in conventional breeding field, but promising clones should be tested in organic field, because of not always previsions proved true.

The genotypes ability format larger size tuber was assessed evaluating large tubers (> 50 mm) percentage in yield. There was significant correlation between amount of large tubers in clones yield in organic and conventional field in 2006. Not significant, but quite high correlation was in 2007, too. Correlation was not significant between trait assessments analysing yield in the same environment two years. The amount of large tubers mostly depends on genotypes environment interaction in particular growing conditions. But evaluating clones in one particular environment ability format large tubers could be detected.

The potato clones tuber's starch content in organic field was higher than in conventional field in 2006 (Table 2). Next year starch content of clones grown in conventional field exceeded starch content in organic field. The reason of this difference was the late blight damages provoked end of growing period approximately week earlier in organic field than conventional. This extra week in conventional field was chance to accumulate more starch in tubers utilising sun energy longer time. Starch content in tubers is mainly determined by genotype. There was found significant correlation between results in different environments and years (Table 3). The genotype's feature accumulate comparatively higher amount of starch could be assessed in any environment.

The assessment of boiled tubers taste did not differed a lot depending on growing conditions in both years. The assessment of clones tubers from organic field was little lower in 2006 and little higher in 2007 than from conventional field (Table 2.). Significant correlation between taste assessment of clones grown in organic and in conventional field was found in 2006 (Table 3). Next year this relationship was not significant. There was not significant correlation between traits assessment in the same environment two year's assessments. This trait depends mostly on genotype expression in different growing conditions including weather conditions.

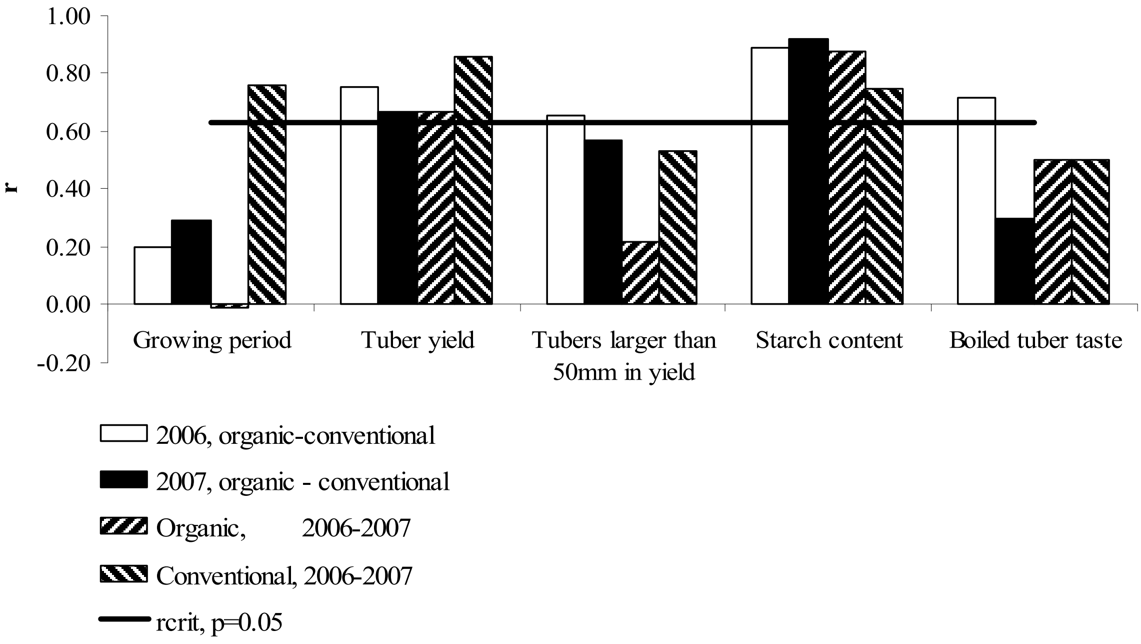

Figure 1. The correlation coefficient (r) between potato clones traits assessment in organic and conventional growing conditions.

Conclusions

The assessment of traits - starch content, tuber yield and partly boiled tuber taste - in organic field significantly correlated with assessment in conventional field. The expression of traits in particular environment depended on genotype; the different growing conditions impact on genotypes trait expression was similar, so differences between genotypes in each environment were relatively similar. Part of selection for breeding potato varieties for organic farming could be done in existing conventional breeding programme.

There was not found significant relationship between traits - length of growing period, ability format large size tubers – assessments in both environments. The traits more determined by environmental conditions preferably could be assessed and selected in particular – organic growing conditions.

The assessment of clone's leaves resistance to diseases has to be continued, the first draw of conclusions verifies, that assessment in particular growing conditions for selection could be preferable.

Particular selection for organic agriculture have to be done in organic field, as selected preferably suitable genotypes with probably acceptable traits in conventional field did not fit to organic conditions as expected.

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TIPSTAR: A USER FREINDLY DECISION SUPPORT SYSTEM FOR POTATO MANAGEMENT

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Tipstar is a decision support system which integrates a crop growth simulation model together with a user friendly GIS based-interface to allow farmers using the system. The simulation model is based upon crop physiological principles and integrates soil, soilwater, nitrogen, weather and crop management into dedicated advice for the farmers regarding potato seed management, crop management and preventing post harvest losses. Examples of the system will be shown and its use in study groups.

POTATO SEED PRODUCTION

THE INFLUENCE OF APHID POPULATION AND VIRUS INFECTION PRESSURE ON SEED POTATO PRODUCTION IN SLOVENIA

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Seed potato production in Slovenia faced a high virus infection pressure in the last decade of the 20th century. The main reasons were the appearance of new strain of PVY virus, high aphid population and high infection sources due to lack of seed exchange.

The acreage of seed potato got smaller and reached about 50 ha per year in the last couple of years (Figure 1). Farmers stopped growing seed potato from different reasons: low yields of susceptible varieties because of very early desiccation, lower quality and consequently no profit in the seed sector (Čergan et al, 2003; Dolničar & Cunder, 2004).

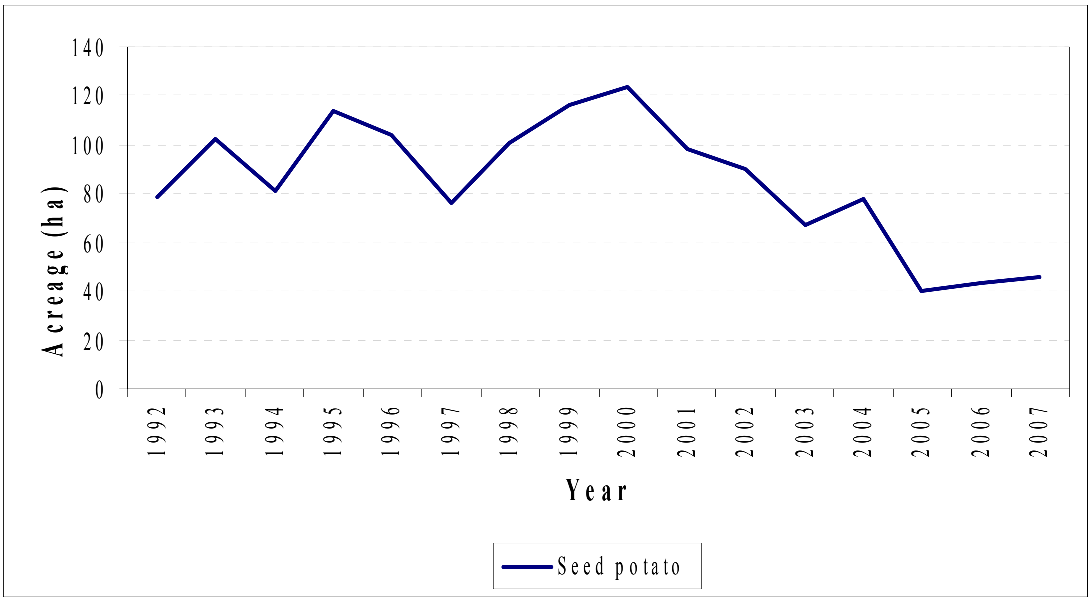

Figure 1: Seed potato production in Slovenia from 1992 to 2007

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The seed quality has improved in the last couple of years due to the reduced aphid population (Table 2 and 3) and introduction of new Slovenian varieties extremely resistant to PVY. Four varieties, Pšata, Bistra, KIS Sora and KIS Mirna, have been registered since 2004. In 1992 there were no elite or higher grades included in Slovenian production with very slow improvement in the following years until 2004. Only the pre-basic or basic seed (SE, E) of Slovenian varieties have been produced since then. The rest of the seed are elite seed material of varieties mainly susceptible to PVY and multiplied once in Slovenia (Čergan et al, 2007).

Table 1: The quality of potato seed produced in Slovenia from 1992 to 2007

Year	S	SE	E	A	B	C
1992	-	-	0	43.08	17.64	39.28
1993	-	-	0.66	18.16	41.61	39.57
1994	-	0.42	2.81	27.19	34.82	34.76
1995	0.99	2.46	0.36	57.29	31.16	7.74
1996	0.97	2.19	5.50	76.71	10.21	4.42
1997	0.44	0.50	2.83	68.51	9.06	14.66
1998	0.36	0.92	1.45	77.53	9.77	9.97
1999	2.73	0.48	4.88	80.33	11.57	-
2000	0.18	1.73	7.83	72.81	13.38	4.07
2001	1.11	1.10	4.55	76.94	7.29	9.06
2002	-	-	9.94	69.67	15.60	4.78
2003	-	-	1.09	90.30	7.29	1.31
2004	0.74	5.64	0.72	89.14	-	3.77
2005	4.20	20.00	5.30	70.50	-	-
2006	1.68	20.71	2.48	66.44	1.95	6.73
2007	1.64	27.33	4.56	66.47	-	-

High aphid population has dropped in the last 15 years, which makes high quality seed potato production feasible. The results of aphid monitoring over the last 15 years in Komenda can be seen in Table 2. Yellow traps on seed potato fields have been used and 14 aphid species which can transmit potato viruses have been monitored. Other aphid species have also been determined, but they are not presented here. The number of aphid vectors dropped from 739 in the season 1996 to 36 in 2003, which was the warmest and driest year ever measured in Slovenia. After that the aphid number started to increase slowly. There has been a large increase in 2007, which is a result of the very mild last winter. This year's winter was also very mild, so we expect further increase of aphid population. We have found similar trends for green peach aphid with sudden increase in numbers in 2007 Čergan et al, 2006, 2007; Južnik et al, 2004).

Table 2: Monitoring of aphid population in Komenda from 1996 to 2007

Species	1996	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	Avg.
Aphis fabae Scopoli	462	55	55	50	47	24	19	28	63	29	45	79,7
Aphis nasturtii Kaltenbach	61	61	63	339	225	115	0	0	0	0	3	78,8
Aulacorthum solani Kaltenbach	0	0	1	0	3	0	0	0	0	0	3	0,6
Brachycaudus cardui (Linnaeus)	0	0	0	0	0	0	0	3	2	6	8	1,7
Brachycaudus helichrysi Kaltenbach	3	97	0	4	27	9	0	2	0	0	14	14,2
Brevicoryne brassicae (Linnaeus)	0	0	0	0	0	0	2	0	0	0	40	3,8
Cavariella aegopodi Scopoli	24	3	0	11	6	11	0	1	0	16	11	7,5
Macrosiphum euphorbiae Thomas	0	1	0	0	0	1	0	6	4	2	16	2,7
Metopolophium dirhodum Walker	0	0	0	0	1	0	1	2	1	0	2	0,6
Myzus ascalonicus Doncaster	0	0	0	0	4	1	0	0	0	0	0	0,5
Myzus certus Walker	0	0	0	0	0	0	0	0	0	0	0	0,0
Myzus pericae Sulzer	20	87	3	42	2	1	2	3	0	1	50	19,2
Phorodon humuli Schrank	168	6	7	29	43	36	1	23	5	17	17	32,0
Rhopalosiphum padi (Linnaeus)	0	2	4	0	10	0	9	11	4	9	9	5,3

Sitobion avenae (Fabricius)	1	9	7	4	2	1	2	4	0	2	7	3,5
Number of all vectors	739	321	140	479	370	199	36	79	79	82	225	249,9
Number of other aphids		399	275	273	323	178	589	191	391	378	269	326,6
Number of all aphids		720	415	752	693	377	625	270	470	460	494	527,6

As we can see from the Table 3 there are also some differences between different locations. Years with the highest population of aphid vectors, of green peach aphids, the lowest population of aphid vectors and the average over 15 years for all three monitored locations are presented in Table 3. The best location proved to be Komenda in the west-northern part of Slovenia followed by Libeliče in the east-northern part and Ivančna Gorica in the southern part of Slovenia. We can see bigger differences between the years. The worst year in the last 15 years was 1996 in Ivančna Gorica, when we counted 1406 aphid vectors. 206 specimens of green peach aphid were found in Libeliče in 2000. Only 14 aphid vectors and no green peach aphid were found in Ivančna Gorica in 2005 which was also a very dry year.

Table 3: An overview of monitoring of aphid population on three locations from 1996 to 2007

	Komenda				Ivančna Gorica				Libeliče			
Species	1998	1996	2003	Avg.	1996	2000	2005	Avg.	2000	2004	2005	Avg.
Aphis fabae Scopoli	55	462	19	79.7	838	270	8	142.6	123	55	29	83.5
Aphis nasturtii Kaltenbach	61	61	0	78.8	486	81	0	76.5	113	0	0	29.9
Aulacorthum solani Kaltenbach	0	0	0	0.6	0	0	0	1	2	0	0	1.4
Brachycaudus cardui (Linnaeus)	0	0	0	1.7	0	0	0	0.5	0	2	2	6.1
Brachycaudus helichrysi Kaltenbach	97	3	0	14.2	0	6	0	4.5	14	1	0	21.6
Brevicoryne brassicae (Linnaeus)	0	0	2	3.8	0	0	0	0.3	0	2	0	0.7
Cavariella aegopodi Scopoli	3	24	0	7.5	5	12	1	5	11	431	1	59.7
Macrosiphum euphorbiae Thomas	1	0	0	2.7	1	0	2	3.1	1	16	6	9.2
Metopolophium dirhodum Walker	0	0	1	0.6	2	1	0	1.2	1	7	5	3.9
Myzus ascalonicus Doncaster	0	0	0	0.5	0	0	0	0.2	0	0	0	1.4
Myzus certus Walker	0	0	0	0	0	0	0	0.1	0	0	0	0
Myzus pericae Sulzer	87	20	2	19.2	26	37	0	10.7	209	5	1	25.9
Phorodon humuli Schrank	6	168	1	32	41	11	0	63.8	13	169	2	28
Rhopalosiphum padi (Linnaeus)	2	0	9	5.3	3	0	2	4.9	0	102	6	28.5
Sitobion avenae (Fabricius)	9	1	2	3.5	6	5	0	9.2	5	32	1	13.7
Number of all vectors	321	739	36	249.9	1408	423	14	325.3	492	816	53	313
Number of other aphids	399		589	326.6	-	525	42	385.3	523	634	138	461.8
Number of all aphids	720		625	527.6	-	948	58	602.5	1015	1450	191	774.6

In conclusion, a number of factors can influence the success of seed potato production. Some of the most important are the use of resistant varieties followed by lack of infection sources together with low aphid population. We can certainly do something about the first and second one but hardly anything about the last one, which is strongly influenced by the environment. Warmer weather in summer can not only reduce seed the potato yield, but also the number of aphids. On the other hand, the mild winters can increase the aphid population as it may be observed in Table 2.

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THE OVERVIEW OF THE RESEARCH AND APPLICATION OF POTATO MERISTEM CULTURE IN ESTONIA

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Abstract

In this paper the overview of the research on potato meristem culture, its application for virus eradication, improvement of variety qualities, and propagation of initial material for the potato seed production and preservation of potato varieties in vitro as a meristem plants is given. In last years, the potato viruses have become more and more important; therefore also the results obtained in the past attract attention. The data about the influence of thermotherapy, the size of meristem and variety on the PVY, PVM, PVX and PVS eradication efficiency is presented. The overview of efficient methods of meristem plant propagation and growing the first tuber generation is given. Some results of meristem clone variability and the exploitation of the phenomenon in practice, the comparison of virus eradicated and improved by means of clone selection seed potato is presented.
Keywords: potato viruses, meristem, multiplication, meristem clones

Introduction

In past years the potato viruses have started to cause serious problems to the producers again. It often happens that seed producers can not certify nor sell their seed potato because of too high occurrence of viruses. Consequently scientists have turned to their earlier studies and findings in order to solve the problems of virus infection fast and efficiently. Research Centre EVIKA in Estonian Research Institute of Agriculture has been engaged in the virus research for 40 years. The first trials were carried out with potato meristem culture for plant regeneration in vitro as there were only a very limited number of studies successfully conducted in the world. When the regenerants were developed it became evident that not all of them were free from viruses. There was a strong need to find out what the virus elimination depended on. When the virus-free meristem plants were established in test tubes there was no experience of using them in seed production practice. This initiated the studies of fast propagation of meristem plants and their usage in seed production. In our studies the yield and morphological characteristics (or deviations) of meristematically eradicated seed potato improved by clonal selection was tested. The research design demanded that the progeny of every meritem was maintained separately as meristem clones. The comparison of variability of meristem clones provided a lot of valuable information to the science and useful results for practice. The results evidenced that meristem clones can differ in their yield capacity, disease resistance and other economical traits even if the morphological characteristics were identical. This phenomenon can successfully be used in improvement of characteristics of varieties. Over 30 years the preservation of potato genetic resources in vitro has been studied in EVIKA as well.

Plant regeneration from meristem

The influence of composition of culture medium, size of meristem cutting, variety characteristics and the time of meristem operation on the effect of plant regeneration was studied. Many basic solutions, various concentrations of mineral salts, plant growth hormones and plant extracts were tested. As a result a modified Murashige-Skoog (Murashige, Skoog, 1962) medium was composed with increased sodium and potassium concentration where growth hormones, microelements and Difco-Bacto agar were added. On this culture medium green plants with stem- and root initials were regenerated from 0,2-0,3 mm meristem cuttings during 4-7 weeks. The speed of regeneration and the number of regenerants depended on variety. We have used the same medium for meristem cultivation of more than 400 different potato cultivars. Depending on variety, 46-87% of tissue cuttings of 0.2-0.3 mm in size regenerated into plants, the regeneration speed and plant quality varied significantly. The best results were obtained with meristems that were operated in April, May or June (Rosenberg, 1980).

The factors influencing the virus elimination efficiency

Our research has shown that some viruses are more easily eradicated by meristem method than other. For example, the 0.1 mm meristems of variety Jõgeva Kollane were 100% PVY-free when regenerated into meristemplants. By increasing the size of meristems up to 0.7 mm, the number of PVY-free plants decreased to 11.7%. The same tendency was observed with other viruses as well - the bigger the meristem cuttings the higher was the occurrence of virus infection. With PVX the results were as follows: meristem size 0.1 mm gave 86% of successful regeneration, 0.5 mm – 12.8% and 0.7 mm provided no virus-free plants. With PVM the results were the following: meristems of the size 0.1 mm regenerated 100% into virus-free plants, meristems of 0.7 mm – only 52%. The most difficult to eliminate was PVS: 0.1 mm meristems provided 6,6% virus-free plants, 0.3 mm meristem – 3.5% and all cuttings bigger than that maintained infected by viruses. Most of the varieties in our trials gave the results similar to Jõgeva Kollane. However, there were some exceptions. For instance, variety Ostbote virus-free regenerant production was at the meristem size 0.2-0.3 mm in case of PVX- 4.3%; PVS-0%; PVM-39% and PVY-8.7%. The heat treatment of green plants prior meristem operation significantly increased the effectiveness of virus eradication. Various heat treatment periods were tested: 4, 5, 6, 7, 8, 9, 10 weeks at the temperature 37-39^{o}C during photoperiod 16 h and 33-35^{o}C during 8 h of dark period. It appeared that the eradication of all 4 tested potato viruses was the most effective when heat treatment was applied 6-8 weeks and meristems 0,2-0,3 mm in size were subsequently operated. Under these conditions 100% of Y, M, S and 91.6% of X-virus free plants were established (Rosenberg, 1980). Further studies with many varieties proved us that success of virus-eradication depends strongly on variety-virus combination. It means, for some varieties it is enough to apply 6 weeks of heat treatment and that some varieties are more easily eradicated from one or another certain virus. Also the PLRV easily eliminated when the green plant thermotherapy was applied and meristems of 0.2-0.3 mm in size were cultivated. As a result of these trials the system of seed potato eradication was created that consists of many cycles (Kotkas, Rosenberg, 1999).

Research of variation of potato meristem clones

In vitro regenerated potato plantlets obtained through virus-eradication procedure were preserved as different meristem clones *in vitro*. The progeny of each meristem was the basis for the meristem clone. The agronomic traits of meristem clones were evaluated and compared in field trials over 3 years. More than 600 meristem clones of 40 varieties were studied during a long time period. The results of our research showed that meristem clones differed by the yield, starch content, some morphological characteristics and disease resistance. For example, the yield of 16 meristem clones of variety Agrie Dzeltenie varied from 32.4 to 51.4 as an average of three trial years. The difference between the meristem clone of the highest yield and lowest yield was 19.1 t/ha (Rosenberg, *et al,* 2007). The previous studies with other varieties evidenced that meristem clones may differ from their starch content in tubers but also from disease resistance. Late blight resistance of meristem clones was tested *in vitro* where the plants were inoculated with pure culture of *Phytophthora infestans* as well as in field conditions. Interesting results were obtained with the variety Bintje, which is known for its susceptibility to late blight. Four meristem clones were studied thoroughly and one was found to be more resistant to late blight than the others. The higher resistance was detected on *in vitro* plantlets and in the field, where less infection was observed on the veins, leaves and on tubers. In the same plots the infection started later. The same meristem clone was superior to the others also for the yield, starch content of tubers and for the uniformity of plant canopy. On variety Ants there was no clear correlation between the susceptibility to late blight and yield. For instance, the most resistant meristem clone gave the yield of 38.1 t/ha as an average and the moderately susceptible clone – 51.8 t/ha (Rosenberg *et al*., 2004). Variety Ants is relatively resistant to late blight itself and therefore the infection by *P. infestans* did not affect the yield remarkably.

The previous studies with other varieties evidenced that meristem clones may differ from their yield, starch content in tubers but also from disease resistance. It is possible to improve the agronomic traits of potato varieties by selecting the meristem clones with suitable characteristics. This phenomenon can be used in seed potato production and in plant breeding.

The multiplication of meristem plants in vitro and in plastic roll

The micropropagation of disease-free plants is presently as integrated part of the seed potato production. There are some methods for propagation of disease-free initial material of seed potato. More widely is used the propagation of potato meristem plants by stem-cuttings *in vitro.*

Plants developed in test tubes are cut into single-node microcuttings and each microcutting is placed onto culture medium in test-tube or another vessel. Various media were tested and the best results were obtained on our modification of Murashige-Skoog medium. On that medium 4-8 cm plants with root initials developed from microcutting within 15-20 days. The regeneration speed and plant quality depended on variety.

The mass propagation of potato plants by *in vitro* method was actively used also by seed producers. For propagation *in vitro* the special laboratory conditions are needed which make this method more complicated and expensive. We started to test simplified and cheap multiplication methods. Mixture of peat and sand was used as rooting substrate in greenhouse. According to the technology created in EVIKA the initial plants regenerated *in vitro* were propagated by single-node-cuttings in plastic rolls in a greenhouse. The cuttings were planted in plastic rolls with peat in a plant arrangement of 2x2 cm. To increase the air humidity and rooting rate the plastic rolls were covered with plastic screen. Approximately two-three weeks after planting the plants regenerated from cuttings were ready to repeat the propagation or to be planted in the field. In our trials plant productivity was affected by the method of multiplication, growing conditions and genotype. The *in vitro* plants had more tubers per plants than plants multiplied in plastic rolls. But the plastic roll method is much simpler, cheaper and therefore easy to use in any greenhouse conditions.

Growing of the first generation meristem tubers

There are several possibilities to grow the first generation of meristem seed tubers. The effectiveness, energy expenditure and the degree of pollution of the environment much differ in each method. It is possible to grow the tubers in winter and in summer greenhouses, plastic or net houses, in the pots, plank beds, in beds and furrows. According to the technology created in EVIKA the plants multiplied in plastic rolls are planted out directly to the field. It is the cheapest, simple and effective way to get more virus-free seed potato and multiply new cultivars. Planting can be done by hand or by planting machine. The first generation meristem plants fields are treated like a normal seed potato field. The aim of cultivation is to get more seed tubers with optimum seed size and avoid the re-infection of the seed. The technology for multiplication of disease-free potato plants in plastic rolls and growing in the field has proven itself well in practice. There were 96 growers in 1987, they had on the open fields 635 000 plants and produced 5.86 million meristem tubers first generation. The average number of tubers per plant was 9.2. Some best results were 31-54 tubers average per plants (Kotkas, Rosenberg, 1999).

The comparing of the seed potato eradicated by meristem culture and by clonal selection

Years ago, when we started with meristem propagation a lot of questions arose like – whether the meristem culture changes the variety-specific traits, is this seed potato tolerant to local climatic conditions and if the disease-resistance will be changed? We compared in the field the three meristematic tuber generations and clonally selected seed potatoes. On the first test year the yield of meristem seed of cv Jõgeva kollane was 75.8%, on second year 36.5% and on third year 15.2% higher than on clonally selected seed potatoes. The number of virus-infected meristem potato plants was 1.5% on the first year, 20.7% on second year and 35% on the third year. Clonally selected seed potato expressed the same values as follows: 39.6; 20.7 and 95%. Meristem seed potato was morphologically true-to-type and had higher resistance to potato late blight (Rosenberg, 1980). These results are proved by many repeated trials. These initial trials brought us to the idea to compare the meristem clones systematically since our meristem clones expressed often better results that potato that was eradicated some another laboratories.

Preservation of potato varieties and meristem clones in vitro

In EVIKA we are preserving potato accessions as meristem plants *in vitro*. All accessions are disease-free and are tested for virus infection for several times. A whole series of experiments were carried out with the aim to observe the influence of different culture medium components, growth conditions and other factors affecting the plants regeneration, productivity and the sub-culturing interval. On the bases of these experiments, the optimal preservation medium and long-term preservation conditions *in vitro* have been developed for many varieties.

The plants regenerated from meristems are initially transferred on a propagation medium containing no growth regulators. In every 3.0-3.5 months the collection is renewed by microcuttings. In every subculture 20 plants per accession are transferred to a fresh medium and the whole collection is duplicated in two storage rooms with different temperature and light regime. The preservation conditions are the following: EVIKA medium without growth hormones, average temperature 4-5°C, photoperiod 16h / 8h. All accessions, preserved *in vitro* have been preserved for the safety reason in the form of tubers, too. At present there are 427 potato varieties, breeding material, land-races and 1026 meristem clones in our gene bank.

Conclusions

In the current paper the brief overview of our research activities is given. In each subsection a large number of trials with many potato varieties were conducted. It was obvious that our seed potato eradication and propagation technology was suitable for most of the varieties. However, there were deviations in speed of meristem regeneration, in amount and quality of developed plants. Also the efficiency of virus elimination was different depending on variety and virus type. Varieties reacted differently to different stages of propagation and growth of meristem tubers. Up to now we have successfully eradicated more than 400 potato varieties from 6 potato viruses. In long-term *in vitro* preservation of meristemplants the varieties behave differently. Further studies are needed an ongoing in this field of research. In the future the detailed studies of variations of meristem clones, their genetic pattern and the possibilities to improve the tolerance of varieties to virus-, bacterial- and fungal diseases are going to be set up. The eradication methods from PSTV need also further studies. We hope that our results can help everybody who studies the potato virus diseases and the methods of eradication. Our findings are also applicable by practical seed potato growers who are aiming for high quality and yield.

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EVALUATION OF MICRO TUBER PRODUCTION IN CONTROLLED ENVIRONMENT – PLANT BIOREACTOR

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Efficient seed production in potato is a prerequisite for successful marketing of varieties. Maintenance breeding focuses on the propagation of healthy and consistent plant material. In-vitro techniques for plant propagation can, in many cases, replace traditional field-based clonal selection. The production of micro or mini tubers under sterile or semi sterile conditions provides the advantage of a controlled environment and season independent production. Propagation rates per plant can be higher than field based systems. Small, uniform tubers provide benefits in handling, storage and transportation. In order to use such benefits NORIKA conducted research on the in vitro production of micro tubers in newly developed plant bioreactors.

Numerous publications describe in vitro tubers production by cultivation of in vitro plantlets on sugar enriched solid media (Wang und Hu, 1982; Pett und Thieme 1982) or using liquid media (ZIV, M., 2002; Hulscher, M., H.T. Krijgsheld & E. Jongedijk, 1996; Akita, M. & S. Takayama, 1994a; Akita, M. & S. Takayama, 1994b; Piao, X.C., Chakrabarty, D., E.J. Hahn & K.Y. Paek, 2003). Commercially available plant bioreactors are of limited size (RITA®- temporary immersion system from Vitropic S.A. / France, PLANTIMA- Systems von A-Tech Bioscientific Co. Ltd./Taiwan) and provide limited options for modification of the cultivation conditions.

Therefore NORIKA developed a unique plant bioreactor system. Reactors were constructed with the ability to control and modify cultivation conditions. The reactor allows for the control of air quality and CO2 concentration. Nutrient supply tanks provide the opportunity to run varied nutrient supply profiles and frequency of flooding of the rhizosphere. Medium may be continuously monitored and replaced if needed. Humidification can also be adjusted within the reactor. Plants in the reactor were subjected different treatments. Nutrient media were modified and various light qualities and photoperiods were tested. The influence of temperature and CO2 enriched atmosphere were determined.

For the tuber production system used it could be shown that the changes in light quality – additional lighting (400 – 500nm) and UVA light - did have an effect on plant vigor. Plants grew shorter and had less formation of plant calli. Tuber production rate was not affected. Photoperiod plays an important role in productivity of plants and tuber initiation. Considering the different modes of photoperiod used one can draw the conclusion that in general the day length of 16 hours at the beginning of cultivation followed by a 12 hour short day cultivation for the induction of tuber formation proved to be successful. Tuberization was enhanced if temperature decrease was combined with photoperiod changes. Temperature lowered to 15°C improved tuber induction. An increase in CO_2 supports photosynthesis and leads to higher yield. The effect of increased CO_2 on plant growth and tuberization and yield was evaluated. However, there is no striking effect of CO2 on overall yield. There is a shift in tuber size. Additional CO_2 led to more tubers with more weight.
For a more efficient use of the described plant bioreactor research will continue.

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NEW PLANTING SYSTEMS FOR PRE-BASIC AND BASIC POTATO SEED PRODUCTION IN SPAIN

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Introduction

Pre-basic and basic seed potato production requires more efficient techniques for obtaining high quality products according to sustainable agriculture criteria. In Spain, where conventional production systems have been employed for the last 25 years, it is especially necessary to apply new methods of production. The objective of this study is to improve potato seed production by applying new and better culture techniques. These are the principal targets:

- Increasing the number of tubers per plant (size class 28-60 mm)
- Obtaining healthier seed potato
- More homogeneous production
- Reducing the number of tubers under 28 mm and over 60 mm
- Improving irrigation
- Improving mechanisation of planting

In summary, the approaches to obtain these objectives are to increase 35-40% density of planting (20 x 80), optimisation of irrigation and the use of whole seed.

Material and Methods

Basic seed potato was produced by micropropagation in APPACALE's facilities. Variety identification by SSR of healthy tubers was performed. These tubers were firstly analysed of *Ralstonia solanacearum*, *Clavibacter michiganensis sepedonicus*, PVY, PLRV, PVS, PVX, PVM and PVA. These non-infected and molecular identified tubers were used for micropropagation. *In vitro* plantlets were grown under glasshouse conditions, and after 3 weeks of adaptation they were transferred to greenhouses and grown in a mixture of turf and sand. Planting frame was 15 x 14 cm (52 plants/m^2). An equilibrated fertilisation and drop irrigation were applied. The objective was to produce tubers of 20-55 mm. This seed, called G-2, is the initial point for its multiplication under field conditions in authorised areas for seed potato production at a height of 1052 m.

Therefore, basic seed of different varieties was planted according to their size class: 28/35, 35/45, 45/55, 55/60.

Furrows of 55-60 cm depth were carried out three days before planting. Then, a star soil separator was used. A high performance of soil separation was achieved by a system of stars with alternate long fingers arranged in spiral configuration for an efficient soil crumbling for an effective cultivation at an ideal depth of 13-16 cm. This system ensures even bed depth across the field of well mixed fine soil and small stones providing good drainage (Figure 1).

Figure 1. Soil separation

Figure 2. Tubers in row.

Finally, a planter with a cup system was employed in pursuit of better levels of planting accuracy.

Soil and water analysis was performed for an appropriate fertilisation. Assessment of tuberisation and virus control according to the regulation of seed potato production in Castilla-Leon were carried out during growing.

Results and discussion

Soil separation was carried out in an area of 8.1 ha. 41.1hours (5.1 hrs/ha) were necessary to prepare the soil. Table 1 show the hours employed in each work.

Table 1. Hours employed in soil separation

Soil separation works	Nº hours	Hours/ha (%)
Effective soil separation	26,6	65
Manoeuvring	8,5	21
Machine adjustment	6	15

Table 2 shows the distribution of costs in terms of time of the 34.5 hours necessary to plant 5.75 ha. (6 hours/ha)

Table 2. Hours employed in planting

Planting works	Nº hours	Hours/ha (%)
Effective planting	10.5	30
Filling hopper	8.4	24
Manoeuvring	4.3	12
Machine adjustment	10.6	31
Filling pesticide product	0.7	2

The number of hours employed in planting and soil separation was higher than the number of hours according to the manufacturer specifications (Table 3). However, heavy rains before planting made these works much more difficult than in a normal situation.

Table 3. Machinery performance.

Task	Hours/ha	
	Theoretic	Real
Planting	2	6
Soil separation	4	5,1

Tubers were planted in a bed with a perfectly separated soil. This enhances plant emergence providing good drainage. The automatic depth control ensures an accurate and even planting depth and homogenous plant emergence. However, this system is difficult to adjust. The cup-system for planting is very precise when tubers of uniform size are used (deviation less than 10 mm) but if no uniform tubers are employed and if the plots have slopes, there is a high number of failures. Planting density can be considerably increased and seed is carefully handled. The planter provides a good relation between the number of tubers planted per ha and the planting frame, but in-row spacing is not very precise (Figure 2).

In-row spacing can be very easily changed because the machine is easy to operate and the values given by the supplier fits the real values of spacing. Plant emergence is 2-3 days later than with the conventional system but it is very homogeneous. Harvest is easier because there are not stones and clods in the bed. In shallow soils the tuber is not very well covered and hoods are irregular.

THE OPTIMAL PERIOD FOR HAULM DESTRUCTION IN ACCORDANCE WITH THE MAXIMAL FLIGHT OF THE APHIDS AND THE SEED FRACTION ACCUMULATION FOR THE SEED POTATO

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Summary

Considering that producing a planting material with high phytosanitary valence takes a lot of effort, after two years of researches that took place in the experimental field from Brasov, tubers with a minimum of virotic infections and corresponding yield have been obtained. This was based upon estimating the optimal period for the haulm destruction, considering the maximal flight of the aphids and obtaining the highest percent of seed tubers, correlated with the sum of thermal degrees during the vegetation period, as two principal criteria which had to be met.

Until now, the moment of haulm destruction of the seed potato cultures was established taking into account the maximal flight of the aphids; in our case, for the semi-early potato varieties (e.g. Ostara), the second evaluation criteria for the haulm destruction, based upon obtaining the highest percent of seed tubers, was used. For the medium late Desireé potato variety and the late Eba variety, the criteria used to estimate the optimal moment for the haulm destruction was the maximal flight of the aphids, which took place before obtaining a satisfactory quantity of seed tubers (30-55 mm).

Key word: haulm destruction, aphid, flight, seed potato

Introduction

Regarding the technology to produce potato for seed, a highly important role for a increased production capacity, have the integrity and the size of the tubers, and also their phytosanitary features and biological quality, which are given by virotic infections.

For seed crops aphid control is essential to minimize the spread of aphid-borne viruses such as leaf roll and severe mosaic. Due to the fact that, on average in each potato seed culture area, the pressure of the vectors is generally high, to be able to produce a high qualitative planting material in concordance with the phytosanitary legislations and also according to the STAS, it is necessary, that in a complex system of agrofitotechique, organization and phytosanitary integrated measurements to take into account the haulm destruction stage. Because of this, the haulm destruction is considered one of the most efficient measurements, in preventing the viruses' transmition from the foliar system to the tubers, but also in limiting the tubers growing in order to obtain an increased percentage of tubers for seeds, from the fraction of 30-55 mm diameter (IANOSI et al., 2002).

In the Netherlands early haulm destruction is the main method of preventing excessive spread of virus diseases in seed potato crops. When potato leaf roll virus was the principal problem, the time of haulm destruction was determined by the flight of *Myzus persicae* (Sulzer), studied with the aid of Moericke yellow water traps (Harten, 1982).

Potato haulm may be destroyed by physical, mechanical, chemical or a combination of both.

Materials and methods

To achieve this goal, there were made determinations regarding the aphids fly monitorising in the experimental field from Brasov, by collecting them in yellow vessels, and as potato cultivar, there were taken into study the following cultivars: Ostara (middle early), Desireé (middle late) and Eba (late), all of them with high phenotypical and genetic variability.

The experience was organized in randomisated blocks, with three variants each in four repetitions, each variant was planted on a surface of 5m^2, at two different planting densities: high density (HD): 63.500 plants/ha, with tubers of 30-45 mm fraction; low density (LD): 53.000 plants/ha, with tubers of 45-55 mm fraction. The biologic category of tubers for seeds was represented by base category, practically free of viruses.

To determine the seed tubers percentage with a diameter between 30-55 mm (diameter mentioned by STAS), it was necessary to find out the weight limits of tubers. In order to do that, WINIGER, LUDWIG, and BROUWER (1976) documentation were consulted; so that at 100 tubers in each cultivar studied there were made biometric measurements concerning the shape, lengh, thikness and tubers weight. These determinations began after about 2-3 weeks from the plants began to grow and continued on the entire vegetation period, from 10 in 10 days, resulting 8-10 harvest stages, depending on the vegetation period of the cultivars taken into study.

The dates obtained show us that the limits of the seed tubers weight variations of 30-55 mm fraction, for Ostara cultivar, are between 17,5 and 102,2 grams, for Desireé cultivar between 38,9 grams and 120,2 grams, and for Eba cultivar between 27,3 and 139,4 grams. The program which was used to calculate the dates is: *SigmaPlot by SPSS.*

Results

Our research, which took place on a period of two years in the experimental field in Brasov, is based on the estimation of the optimal haulm destruction period depending on the achievement of the two main criteria and that is the maximal aphids fly period and a increased percentage of seeds tubers, depending on the degrees of thermal sum accumulated from the moment of tuber plantation up to the last harvest stage.

Concerning the fact that the main criteria to establish, presently the moment for haulm destruction of seeds potato culture, is the maximal aphids fly period, in our case, for the middle late cultivar (Ostara), characterised by a relativly short vegetation period, 75-85 days, is available the second criteria to apreciate the haulm destruction, based on the maximum seed tubers percent achievement.

This recommendation is justified due to the dates obtained, at the level of the culture with the planting densities (63500 plants/ha and 53000 plants/ha) (fig. 1). It is shown that, to achieve the maximum seed cuantum, on 30-55 mm, it is obtained before the maximal aphids fly period, which takes place at a cumulated value of the temperatures degrees of 1200ºC.

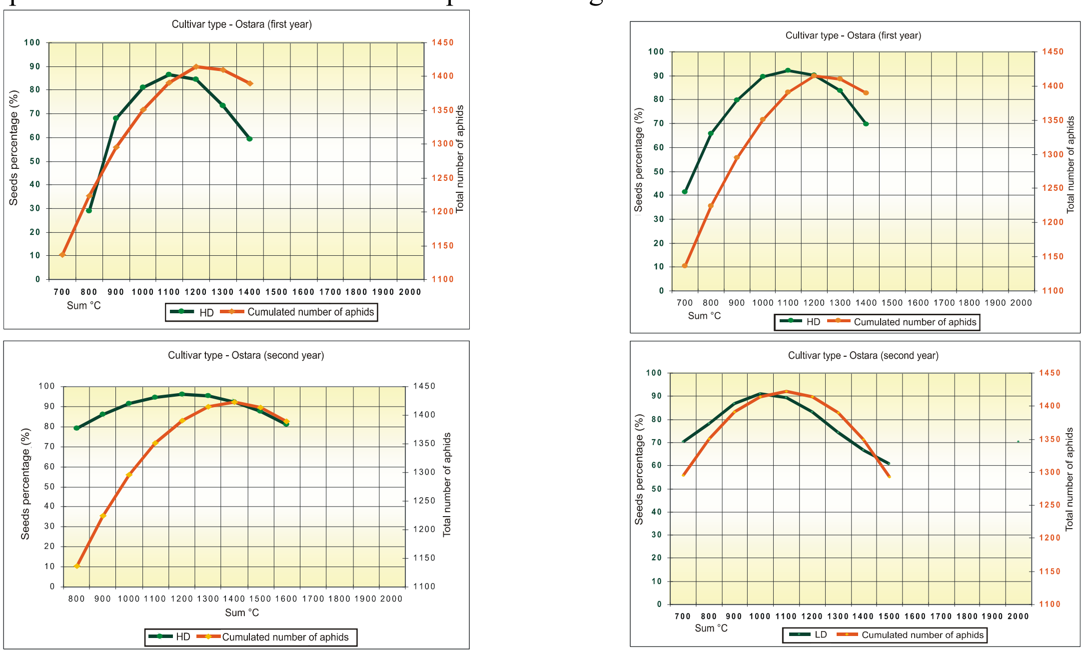

Fig. 1. The maximal aphids fly and maximal percentage of seeds tubers - Ostara cultivar

For a superior quantitative and qualitative production of seed tubers, the optimal perios for haulm destruction can be estimated when the sum of temperature degrees cumulated reaches a value between 950-1250°C, depending on the climatic conditions of the certain years. Calendaristically speaking, these values are according to the date 10-20 July, respectively after 50-65 days from the plant appearance. The aspect noticed determines us to consider that haulm destruction is justified to be achieved after the criteria of maximal seed production achieved, which can be done before the maximal aphids fly period, in this way the virotic infections of the planting material being , significally, decreased

For Desireé, middle late cultivar, due to its longer vegetation period, achieving a superior qualitative production of seed tubers, was possible only after reaching a level of temperature degrees of over 1300°C, in all the two years studied (Fig.2). Due to this fact, the criteria used to estimate the optimal moment to haulm destruction is the moment of the maximal aphids fly, which takes place before obtaining a satisfying seed tuber quantity (30-55 mm). To obtain a qualitative production, from the phytosanitary point of view, the optimal moment for haulm destruction based on the maximal aphids fly period, can be estimated as being the last decade of July, when the sum of the thermal degrees reaches 1150-1200°C.

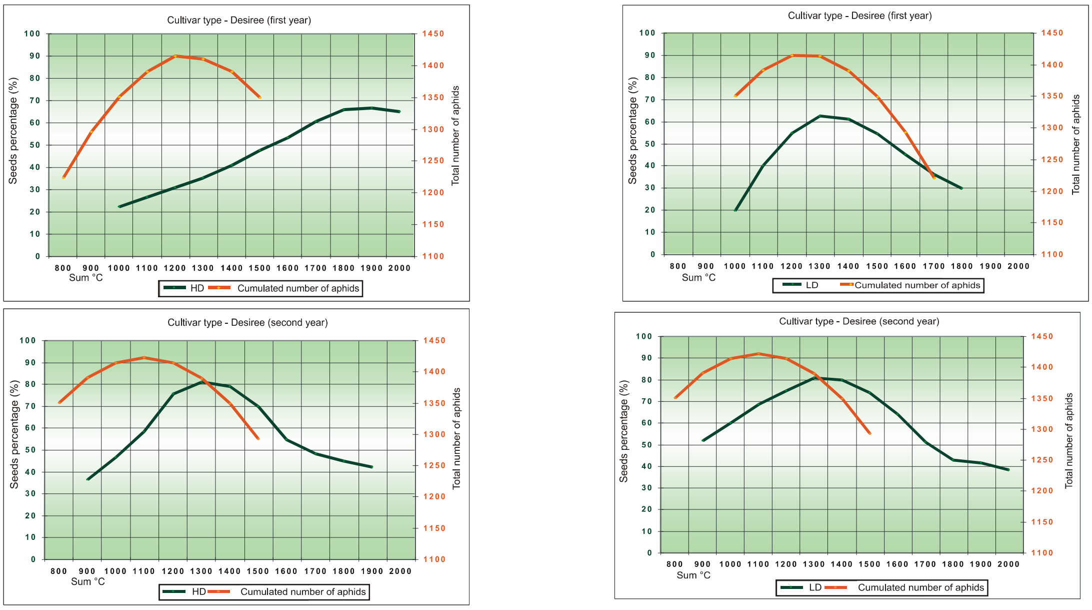

Fig. 2. The maximal aphids fly and maximal percentage of seeds tubers - Desiree cultivar

The criteria used to estimate the haulm destruction for late cultivar Eba, is in this case the maximal aphids fly period, due to its vegetation period of over 120 days, the accumulation of an appropriate quantitative of tuber production, achieved on temperatures over 1700°C, in the first but also in the second year of study for both of the planting densities.

So, the optimal period for haulm destruction depending on the maximal aphids fly period, is estimated to be the last decade of July in the case of the first year, and the end of the second decade of July in the case of the second year of study (Fig. 3).

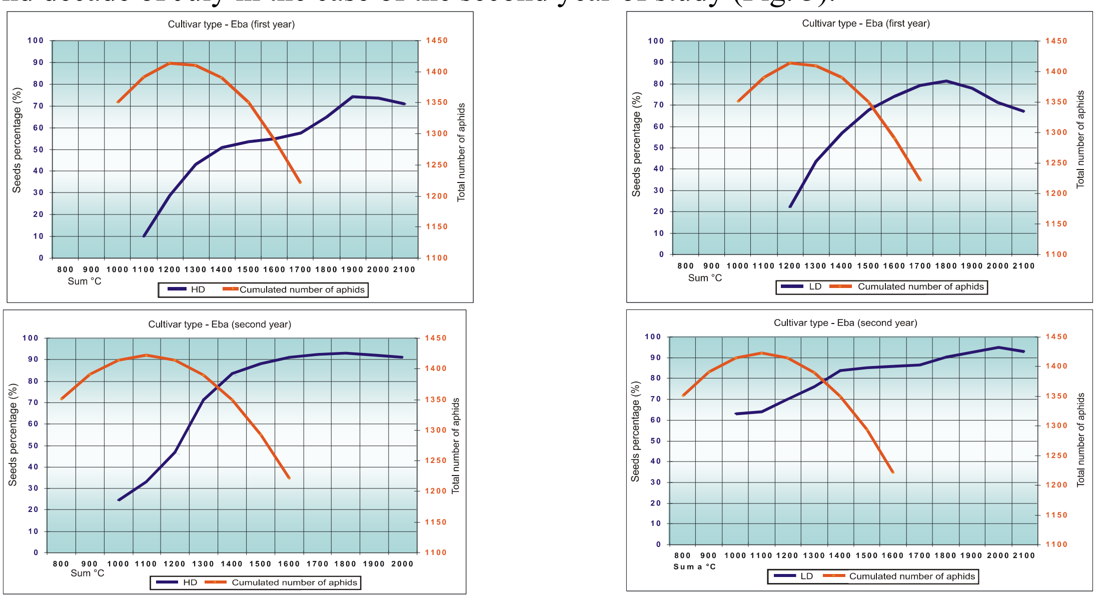

Fig. 3. The maximal aphids fly and maximal percentage of seeds tubers - Eba cultivar

Practically, in this case it can be recommended as optimal period to destroy the stalks, when the sum of accumulated thermal degrees reaches the value of 1100 - 1300°C.

Conclusions:

- The haulm destruction is necessary to reduce late blight and virus spread, to reduce interference at harvest, to improve skin-set and to control tuber size.
- The aspect noticed in the case of Ostara cultivar determine us to consider that haulm destruction is justified to be achieved after the criteria of maximal seed production obtained, achieved before the maximal aphids fly period, decreasing in this way, significally,the virotic infections of the planting material.
- In the case of the two cultivars, middle late Desireé and late Eba, the optimal haulm destruction is established after the criteria of the maximal aphids fly period, due to the high susceptibility of plants to infection and obtaining superior qualitative production, but not quantitative, because the vegetation periods are more extended.
- The possibility to obtain satisfactory production under this aspect is low, because the tubers aren't mature from physiological point of view. That is why the planting period has an important role; it has to be made as early as possible for seed culture, measure which contributes to a satisfactory seed production accumulation up to the optimal moment for haulm destruction.

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POTATO SEED PRODUCTION IN TATARSTAN

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Disease-free seed potatoes production has been begun in Tatarstan since the middle of eighties. Tatarstan is the region of Russian Federation located in middle Volga 800 km east of Moscow. For a long time virus-free seed potatoes introduction was restricted by fast virus re-infection in field conditions. Virus re-infection grow up to 50-60% during 2-3 years was detected at this time. Mane virus infection reservoir in Tatarstan conditions was detected as a virus infected potato plants inside seed plots. High number of aphids causes large scale virus transmission from infected plants to healthy in potato fields. According our data weeds are not really dangerous reservoir of potato viruses in Tatarstan conditions. Potato virus Y (PVY) was demonstrated the most widespread between other potato viruses in Tatarstan fields. Large scale of PVY widespread in middle Volga region can be explained by transmitters predominance. The continental climate of Tatarstan has appeared unfavorable for reproduction of a peach aphids (*Myzus persicae* Sulz.) the most dangerous transmitters of PLRV, PVY, PVM.

Healthy seed potato production is based on knowledge four components of virus epidemiology: the causal viruses, their insect vectors and plant hosts and the environment in which these interact.

Long-term survey of virus epidemiology gave possibility to produce healthy potatoes in Middle Volga region.

Fast virus re-infection had been caused "inside" infection from virus infected *in vitro* potato plants and "outside" infection transmitted by aphids for many years before virus detection and controlling measures was improved. "Inside" infection was eliminated by introducing molecular virus detection methods, such as enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). Potato plants testing for PVY, potato virus M (PVM), potato virus X (PVX), potato leaf roll virus (PLRV) and potato spindle tuber viroid (PSTV) have been introduced in Tatar Institute of Agriculture (TIA) since the late of 90-s.

"Outside" virus infection is transmitted by aphids from infected potatoes, weeds and trees. According our data weeds and trees do not really dangerous sources of virus infection in Tatarstan condition. Similar results was demonstrated in Vlasov (Vlasov, 1974) study.

For a long-term survey, area in Middle Volga region of Russia was selected at Sokury (Tatarstan, 30 km to the south from Kazan the capital of Tatarstan). Yearly surveys were made at Sokury between 1993 and 2005.

Aphids were caught in the water-filled yellow trays (YWT) (Moericke, 1955). Trays were placed along and inside seed potato fields. Trays were used from the time of tuber planting until haulm destruction (usually between May and August). Aphids from YWT were collected twice weekly and were preserved in 70% ethanol until being identified and counted. All species were identified with the key of Ivaniuk at al. (2005).

The total number of aphids caught in YWT during yearly survey, the individual and total number of virus vectors and the cumulative vector intensity was calculated. The cumulative vector intensity is an index that describes vector abundance and their propensity to transmit potato virus Y (Irwin and Ruesnik, 1986). The propensity is the highest for the most efficient vector and the lowest of the least efficient vector.

In this study, the following vector propensity values were used, based on the results of: *Myzus persicae* Sulzer 1.0, *Macrosiphum solanifolii* Ashm 0.10, *Acyrthosiphum pisum* Harris 0.05, *Aulacorthum solani* Kaltenbach 0.4, *Aphis nasturtii* Kaltenbach 0.42, *Aphis fabae* (Scop.) 0.1, *Aphis frangulae* Kaltenbach 0.42 (Siegvald, 1984; Ivaniuk at al., 2005). Cumulative vector intensity for the season was calculated by accumulating species-specific vector intensity values at each trapping date.

Virus infection of potato plants was determined during visual inspection and collected leave testing with ELISA. Virus infection of progeny tubers was assessed during post-harvest inspections.

Tubers were selected and sprouted in laboratory. The sprouts were tested with ELISA (one tuber – one sample). Bioreba ELISA test kits for PVY, PVM, PVX and PLRV detection was used.

During the long-term experiment between 1993 and 2005 years the total annual number of aphids caught in YWT varied from 82 to 5000 (Figure 1).

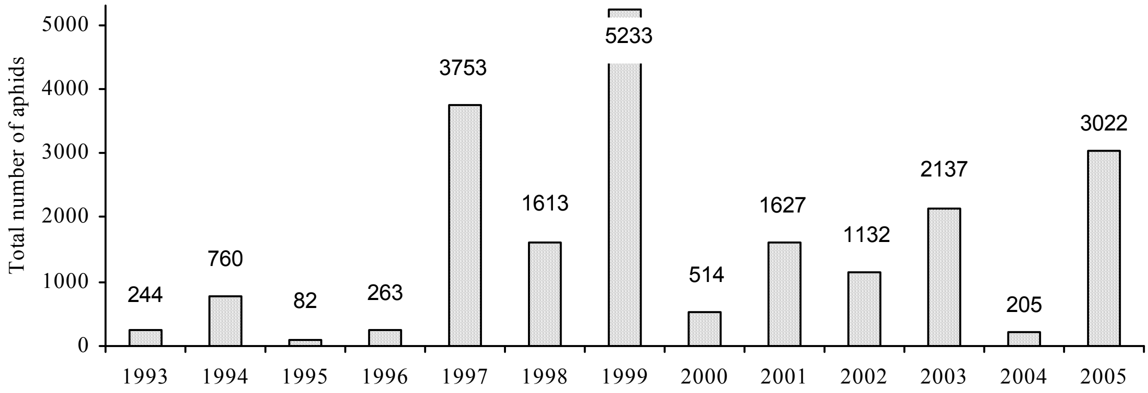

Figure 1. Total annual number of aphids in Sokury (Tatarstan, Russia) 1993-2005.

The total number of *Myzus persicae* Sulcer the most dangerous transmitter of persistent PLRV and non-persistent PVY, PVM was not significant and varied from 0.5-5. The total number of *Aphis nasturtii* Kalt, *Aphis frangulae* Kalt vectors of PLRV, PVY and PVM was up to 684 and 784 (16% from total number of virus vectors), respectively. PVY transmitting *Aphis fabae* (Scop.) and *Acyrthosiphum pisum* Harris the total number was the highest up to 2407 (up to 57%) and 2070 (up to 44%). Thus the biggest amount of vectors is able to transfer PVY, then PVM and PLRV was detected in Tatarstan conditions.

The influence of environmental factors on aphid population dynamic, total number of aphids and cumulative vector intensity was studied. Temperature regime of May and early June determine directly the date of aphid emigration flight. There was significant positive relationship between accumulated air temperatures and the date of aphid emigration flight. The earliest aphid emigration flight from primary host was observed by the first decade of June. No relationship was observed when cold temperature with night frosts came back during spring and/or early summer time. Sharp temperature falls have long-lasting negative effect for the start of aphid distribution.

Thus, two significant environmental factors play a key role in aphid population development was revealed for Tatarstan conditions. Cold temperature was pernicious for insects during multiplication on primary host usually took place in May. Rains and strong wind restricted aphid emigration flight from primary host usually took place in June. Temperature falls following warm is frequent event in Tatarstan in May. The aphid population size directly depends on the number of overwintered aphids, which survived spring cold and was able to reproduce progeny. Therefore the cold temperature in May significantly decreased the total number of aphids.

The limiting role on the aphid population development plays the environmental conditions during winged forms emigration flight too. Long-term rains and strong wind prevent winged insect flight from primary host to new host. The progeny of overwintered aphids starved during long-term stay on primary plant. They have been washed away by rain and/or gone by wind when the June was unfavourable. The first generations losses in June play a crucial role in total number of aphids and drastically reduce insect population.

During the long-term survey between 1993 and 2005, the total number of aphids varied with no cycle and mostly depended on June-July weather conditions. The most important was availability of favourable temperature (18-23°C) and humidity. Droughts have reduced the number of vectors usually. Host-plant presence and its juicy had the influence on aphid population dynamics.

In favourable environment conditions were detected two aphid flight peaks during third decade of June to first decade of July and during second decade of July to third decade of July, respectively. There was positive relationship between favourable environment conditions and intensity of aphid flight peaks.

The critical threshold of total aphid number means cumulative vector intensity caught in YWT from the beginning of cropping season. The critical threshold equal 50 individuals of *Myzus persicae* Sulzer. The forecast for the risk of virus spread and determination of haulm destruction dates were empirical and based on cumulative vector intensity in YWT. Haulm destruction date is recommended within 12 days after the critical threshold was exceeded.

The total number of aphids and cumulative vector intensity was law and the critical threshold was not exceeded in 1993, 1995, 1996, 2000, 2002, 2004 years (Table 1).

The total number of aphids and total number of virus vectors caught in YWT then critical threshold was reached varied between 438-1280 and 338-782 (61-96%), respectively (Table 1).

Table 1. Total number of aphids, total number of virus vectors, critical threshold dates in Sokury (Tatastan, Russia) 1993-2005.

Year	Total number of aphids	Critical threshold date	Total number of aphids at critical threshold date	Total number of virus vectors at critical threshold date	
				Number	%
1993	244	Total number of virus vectors below critical threshold			
1994	760	Early first decade of August	748	467	62
1995	82	Total number of virus vectors below critical threshold			
1996	263	Total number of virus vectors below critical threshold			
1997	3753	Early third decade of June	550	338	70
1998	1613	Second decade of July	438	351	80
1999	5233	First decade of July	493	360	73
2000	514	Total number of virus vectors below critical threshold			
2001	1627	Early second decade of July	809,5	525	65
2002	1132	Total number of virus vectors below critical threshold			
2003	2137	Early third decade of July	1280	782	61
2004	205	Total number of virus vectors below critical threshold			
2005	3022	Early third decade of July	462,5	443	96

The risk of virus spread was low in 6 years (1993, 1995, 1996, 2000, 2002 and 2004). The critical threshold was reached late (at first decade of August) in 1994 and the risk of virus spread in seed potatoes fields was low too. The total number of aphids and cumulative vector intensity was high and the critical threshold was exceeded early in 1997, 1998, 1999, 2001, 2003, 2005 years (Table 1). The mentioned situation is unfavourable for seed potatoes growers. There was not reached economically significant tuber yield at the early haulm destruction date.

Direct relationship were found between seed potatoes re-infection and the total number of aphids in YWT, the total number of virus vectors, cultivars resistance and the initial level of crop infection. The initial level of potato infection plays key role in aphid-borne virus spread in Tatarstan. The essential in the high grade seed potatoes production in high level of virus vectors area is to avoid infected tuber planting. This requires growing one seed potatoes reproduction at least 1-2 km away from other reproduction.

The presented date has shown possibility of high grade (virus-free) seed potatoes production in Middle Volga region there virus vectors intensity might be able to influence virus spread in same years.

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TEHNOLOGICAL SOLUTIONS OF CULTIVATION AND PHYTOSANITARY CONTROL, ECONOMICAL EFFICIENTLY, USED FOR PRODUCING PRE-BASIC CLONAL MATERIAL TO POTATO

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Key words : potato, seed, technology, quality, efficacy.

ABSTRACT

The value of the PREBASE material is very important: must be taken a maximum precautions to prevent early infections and to maintain the multiplication potential coefficient, with reported tendency to increase profitability of the material rised from „in vitro" cultures. The protection at the level of minitubers must be very intensive. Therefore the tunnels „insect proof" implementation, against aphids, must be watched as a very efficient solution in conservation of the phytosanitary clonal material, and financial - very economic.

Technological variants of cultivation and phytosanitary control applied in clonal field and in protected areas type tunnels 'insect proof', follow to increase the efficienty of applied chemical fertilizers, the improvement of plants nutrition in the first levels of vegetation, and the increase of multiplication rate, too.

The behaviour of experimented varieties, seed productions, achieved multiplication rates are characteristics determinated directly by variety, culture conditions, NPK fertilization way, planting material size, applied cultivation and phytosanitary control technology and existed climatical conditions.

INTRODUCTION

The objectives of this research are centred on quality and quantity perfection in producing of pre-basic potato seed, through application the efficient phytosanitary cultivation and control technological sequences and development the process of potato planting material regarding the first clonal links through the implementation in clonal fields of protected areas with net, „insect proof", against aphyds, based on technological phitosanitary cultivation and control variants.

In this work paper are presented applicated phitosanitary cultivation and control technologies, the quantitative and qualitative results of experimental alternatives obtained in years 2004 and 2005.

Experimenting climatic and pedological conditions. **The Giurgeu Country** is situated in central – west part of Oriental Carpathian range, along Mures river, in north – south about 75 km length and in east – west about 30 km width.

In process of seed potato production the cultures are located in „closed areas" conditions with favourable climatical conditions, with a later and reduce appearance of aphyds. Also, are excluded contaminated ares with black scab (*Synchitrium endobioticum*) and *Globodera spp.* nemathods.

In the closed areas the spring is short, cold and frequent rains. The climate is humid and cool, the biggest quantities of precipitation are falling down in June, July and August, with a high frequency of rainy days in June and July, which limit aphyids fly. Multiannual average of precipitation is 700 l/m^2. Annual average temperature is 5,6^0 – 7,5^0 – 7,8^0 C, and the highest monthly average temperature is 16 – 19^0 C. The maximum frequecy of aphyds summer flying is situated, on average, in the 2nd and 3rd July decade. In these pedoclimatic, „closed areas", conditions were located the studied experimental alternatives between 2004 – 2005, which were iside of clonal fields A, B from Clonal Centre for Planting Potato Material, Lăzarea, Harghita county, departament which takes part to NIRDPSB Brosov, region characterized by specific ecological conditions ask for producing the potato pre-basic seed, where the fields and laboratory is in use since 1967.

Experimental variants from 2004 – 2005 were located on a land which was maintained as a cultivated field in previous year, inside of clonal fields A and B (Căpâlna body) from the Centre for Producing Potato Clonal Material - Lăzarea, Harghita county, departament of NIRDPSB Brasov, situated about 1100m altitude over sea level.

The predominant soil, where took place the experiment, is specific (pale brown), extremely deep on emaciated earths, earthen / moderate earthen. The aspect of soil is normal, the surface is covered with stones in 10-20% rate. A deeply profile A-El-Bt-R, in 0-50 cm layer the porousity is very big, airing porousity is moderate, edaphic volume is big, at the surface (Ap) the soil reaction is strongly acid, capacity of cathionic exchange (T=13,5) is moderate , humus and total nithrogene content and mobile phosphorus are moderate, too, in mobile potassium is high.

Year 2004 presents very closed values to MAA (multiannual average), and annual average temperature was 5,9^{0}C. The sum of precipitations in 2004 was 580,5 l/m^2. The total abundance of captured aphyds from June to August was 395 individuals from 29 varieties with 110 captured individuals, eudominant species being *Aphis fabae* Scop (27,84 %). *Mizus persicae Sulz* having a number of 5 captured individuals and a subrecedent domination of 1,26%. The most intens fly of aphyds is in the second decade of July (101 capturated individuals).

In 2005 there is a differece in May +2,1^{0}C towards MAA, the other temperatures being very closed to MAA. The annual average temperature was 5,1^{0}C. The sum of precipitations in 2005 was 653,4 l/m^2. The total abundance of captured aphyds from June to August was 217 individuals from 27 varieties with 107 captured individuals, eudominant species being *Aphis fabae* Scop (49,30 %). *Mizus persicae Sulz* wasn't captured in this period of time.

BIOLOGICAL MATERIAL AND RESEARCH METHOD

Biological material used in experimentally years 2004-2005 is comes from „in vitro plantlets", microtubers and minitubers obtained in „insect proof" green houses.

a. **Roclas** is a Romanian variety, aproved in 1994, created by N.I.R.D.P.S.B. Brasov.
b. **Desiree** is a Dutch half- lated variety, having the genealogy : URGENTA x DEPECHE, brought in Romania in 1971.
c. **Sante** is a Dutch variety, created by AGRICO firm from Holland, brought in Romania in 1994.

In experimentally years 2004-2005 was studied and experimented a new technology, an improved phytosanitary cultivation and control technology, using "insect proof" tunnels (net), protected spacies against aphyds -the main vectors of contamination with viruses (field culture), a technology which had the purpose: minitubers cultivation and obtaining the pre-basic (clonal) material for next links.
In experimented field was located an bifactorial experience type 3 x 2. Research alternatives were 6. The number of repetition were 3. Experimental alternative have : 22m in length x 3 m width = 66m^2. We was use 600 minitubers / alternative, each minitubers was the weight between 8,60 -10,00 grams.

Research method and experimented alternatives:

A factor: potato variety with 3 degrees: **a_1**- Roclas **a_2** – Desiree **a_3**- Sante

B factor : crop conditions and phytosanitary control : fertilization with N_{90} : P_{90} : K_{90} kg./ s.a/ha, superphosphate (20 kg s.a./ha) + amendments (4,5 t/ha) + virotical control through covering "insect proof" tunnels (net) with 2 degrees:

- **b_1** - **crop conditions**: autumn ploughing with incorporated meadow, fertilization with N_{90} : P_{90} : K_{90} kg./ s.a/ha + chalky amendments (4,5 t/ha) + superphosphate (20 kg s.a./ha), with virotical control through covering "insect proof" tunnels(net) .
- **b_2** - **crop conditions**: autumn ploughing with incorporated meadow, fertilization with N_{90} : P_{90} : K_{90} kg./ s.a/ha + chalky amendments (4,5 t/ha) + superphosphate (20 kg s.a./ha), without virotical control through covering "insect proof" tunnels (net) .

Observations and determinations. The followed phenological dates are: planting, rising, breaking vegetation. During vegetation were observed phenophases and uniformity of potato field. Were followed the number of rising plants and were done visual obsevations on virus groups (leafroll, streak, grave and low mosaic) for noticing the number of contaminated plants, and establishing the final number of plants / alternative. The elements of production capacity, determinated by counting and weighing were: the number and weight of seed, table and small tubers / experimental alternative. Was determined the total production of tubers and the number of tubers at nest. Was establised the seed tubers rate, the tubers weight average, rised plants rate and healthly plants. Determination of phytosanitary plants state was done in laboratory by ELISA test. For experimental alternatives with "insect proof" tunnels, was mainly followed the phytosanitary state (qualitative state) and multiplication rate.

RESULTS AND DISCUSSIONS

From <u>total production</u> in experimentally years 2004 -2005 is obvious the higher level of harvested tubers to experimental alternatives without 'insect proof'tunnels, comparative with 'insect proof' tunnels experimental alternatives, at all varieties. **Roclas (a factor) i**s obtained the highest level of total production in 2004, respectively a total production 185,74kg / 'insect proof'tunnel (**b1** factor), and a total production 195,03 kg / experimental alternative (**b2** factor). **Desiree (a2 factor),** the level of total production was 134,41 kg/ tunnel (**b1** factor) and 152,01 kg / experimental alternative (**b2** factor). **Sante (a3 factor),** have in 2004 the lowest level of total production between experimented varieties, with 91,00 kg /tunnel (**b1** factor) and 130,58 kg / experimental variety (**b2** factor)(figure 1).

In this way **Roclas (a1 factor)** has the highest level of total production in 2005, respectively a total production 172,00 kg /"insect proof" tunnel (**b1** factor) and a total production – 180,60 kg / experimental alternative (**b2** factor) . **Desiree (a2 factor),** the level of total production was 124,00 kg/ tunnel (**b1** factor) and 131,44 kg / experimental alternative (**b2** factor). **Sante (a3 factor),** is noticed the level of total production –144,00 kg /"insect proof" tunnel (**b1** factor), respectively 154,08 kg / experimental alternative (**b1** factor)(figure 1).

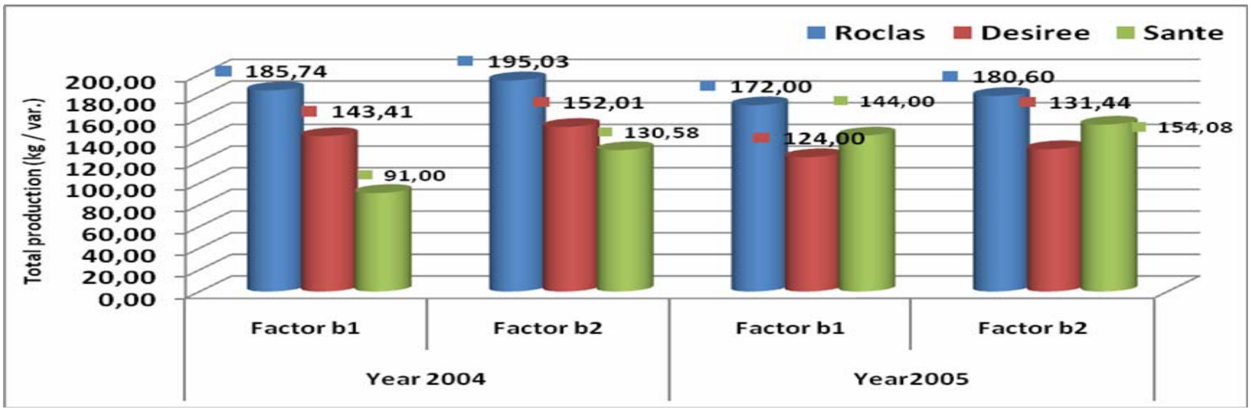

Figure 1. Variation of the total production / experimental alternative in 2004 – 2005.

<u>The total tubers number (multiplication rate)</u>(figure 2). In experimentally year 2004 the **Roclas** variety (**a1** factor) obtained the highest level of experimental averages, respectively a total number of **4385 tubers** /"insect proof" tunnels (**b1** factor), with multiplication rate 1:7,31 and a total number of **4536 tubers** / experimental alternative (**b2** factor), with multiplication rate 1:7,56. Tthe level of the total harvested tubers **Desiree** variety (**a2** factor) is **2980** tub. / tunnel (**b1** factor), with multiplication rate 1:4,97 and **3354** tub./ experimental alternative (**b2** factor), with multiplication rate 1:5,59. The **Sante** variety (**a3** factor) obtained the lowest level of total harvested tubers, from experimented varieties, with **2328** tub. / tunnel (**b1** factor), with multiplication rate 1:3,88 and **2432** tub. / experimental alternative (**b2** factor), with multiplication rate 1:4,05.

In experimentally year **2005** the **Roclas** variety (**a1** factor) has the highest level of total number average including the harvested tubers in 2005, respectively a total number of **4520** tub./ experimental alternative (**b2** factor) with multiplication rate 1:7,53 and **4385** tub./ experimental alternative (**b1** factor), with multiplication rate 1:7,05. To **Desiree** variety **(a2 factor)** experimental average level of tubers total number was **2630** tub. / tunnel (**b1** factor), with multiplication rate 1:4,38 and **2924** tub./ experimental alternative (**b2** factor) with multiplication rate 1:4,87, this variety having in 2005 the lowest level of harvested tubers from experimented varieties. At **Sante (a3 factor)** is noticed **'insect proof'** alternative with a total number of **3000** tubers / tunnel (**b1** factor) with multiplication rate 1:5,00, respectively **3325** tubers / experimental alternative (**b2** factor) with multiplication rate 1:5,54.

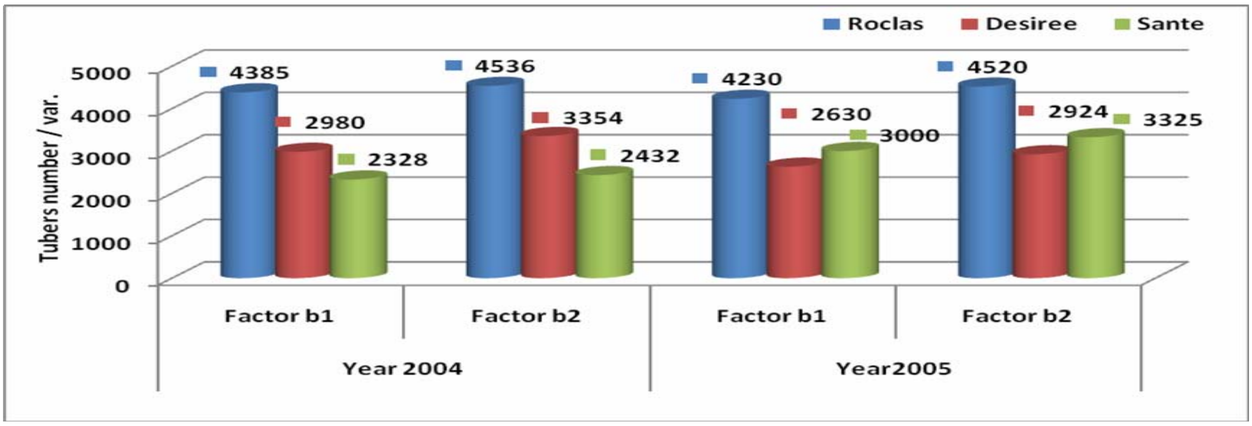

Figure 2. Variation of the total tubers number / experimental alternative in 2004 – 2005.

Althought the level of productions and multiplication coefficients obtained to the bouth experimental variants (factor b1 and b2) are rised, however in case of covered variants with „insect-proof" net (factor b1), we can see smaller values comparative with factor b2, this fact thanks to the shadow coefficient (15%) of „insect-proof" net, respectively less intense solar radiation insaide of „insect-proof" tunnels.

Phitosanitary state (tabel 1). In experimentally years 2004-2005 phytosanitary state of material was very well, just in case of Desiree variety, found virotic positive probes, afther ELISA test.

Tabel 1. ELISA test results from experimental years 2004-2005.

Year	Variety	Variants	1st ELISA test (July) Sample number	2nd ELISA test (November) Sample number	Potato virus		
					PLRV – PVA	PVX – PVS	PVY - PVM
2004	**Roclas**	Factor b1	-	200	-	-	-
		Factor b2	200	200	-	-	-
	Desiree	Factor b1	-	200	-	-	-
		Factor b2	200	200			1
	Sante	Factor b1	-	200	-	-	-
		Factor b2	200	200	-	-	-
2005	**Roclas**	Factor b1	-	200	-	-	-
		Factor b2	200	200	-	-	-
	Desiree	Factor b1	-	200	-	-	-
		Factor b2	200	200	-	-	1
	Sante	Factor b1	-	200	-	-	-
		Factor b2	200	200	-	-	-

Qualitative and quantitative results obtained in experimental years demonstrate the viability and utility of proposed technology, the implementation of „insect proof" tunnels in pre-basic fields, assuring an efficient healthy protection of minitubers while reducing the costs and the number of multiplication years used in actual system for pre-basic seed.

ECONOMY, STATISTICS

CHALLENGES IN POTATO PRODUCTION IN THE RUSSIAN REPUBLIC OF KARELIA

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SUMMARY

A three-year (2007-2009) collaborative project was set up to identify the most important problems in potato production in the Russian Republic of Karelia and to increase the potato yields and improve the crop quality. In the first year of the project, the effect of cultivars from different origins and the type of fertiliser on diseases was investigated in a field experiment. The main disease problems were caused by *Rhizoctonia* stem canker and black scurf and potato viruses. *Rhizoctonia* infection was both soil-borne and in the case of Russian cultivars also seed-borne.

INTRODUCTION

Russian Federation is the second biggest potato producing country in the world (FAO 2008). Over 90 percent of Russian potatoes are grown on household plots and private farms, with average yields of 13 t/ha. In the Russian Republic of Karelia in 2006, potato was cultivated on 9 500 ha of the 969 000 ha of agricultural land (Russian Federal State Statistics 2006b). Potato is not only used as staple food in human consumption, but also as fodder for cattle in milk and beef production.

In the Russian Republic of Karelia potato yields are low (less than 10 t/ha) compared to those in Finland (30-60 t/ha), although Finland and Karelia have similar climatic and soil conditions for potato production. The low yield is due to a complex of factors as well as to the fact that potato is a vegetatively reproduced crop rapidly accumulating infection during the reproduction process. The available seed material is often of poor quality and infected by pathogens, as the volume of certified seed potato production is inadequate (Anisimov 2007). Pests and diseases are a major problem in Russian potato production causing annual losses of 4 million tonnes (FAO 2008). However, it is not known which diseases cause the greatest yield losses in the Republic of Karelia. Furthermore, organic and other compost-based fertilisers are commonly used in potato production. In 2006, 15 t/ha of organic fertiliser was used for potato, whereas the amount of synthetic fertiliser was only 213 t/ha (Russian Federal State Statistics 2006a). Use of organic fertiliser is problematic, as the release and demand of nitrogen does not coincide. Potato crop requires a sufficient amount of nitrogen in the beginning of the growth, whereas nitrogen is usually released from organic fertilisers in the end of the growing season. In contrast, industrial fertilisers allow dosing of nitrogen and other nutrients in a cultivar-specific manner.

A three-year collaborative project funded by EU (Intereg/Tacis) was started in 2007. The project is led by MTT Agrifood Research Finland Ruukki. The major cooperation partners are NorTech Oulu/ University of Oulu, University of Petrozavodsk, Finnish Seed Potato Centre Ltd, ProAgria Rural Advisory Center and Finnish Food Safety Authority Evira. The general aim of the project is to increase the potato yield and improve the crop quality and thereby guarantee self-sufficiency in potato production in the Republic of Karelia. To achieve this, the subsequent objectives are 1) to identify the most important diseases in potato production in the Republic of Karelia, 2) to assist in the maintenance of healthy seed material and production of basic seed and 3) to determine the effect of the organic fertiliser on the growth and yield of potato. In addition, information is gathered on the occurrence of quarantine pathogens and pests such as ring rot bacteria (*Clavibacter michiganensis* subsp. *sepedonicus, Ralstonia solanacearum*) and potato cyst nematodes (*Globodera* spp.) in order to assess a thread these diseases pose. As samples of soil, leaves and tubers are imported from the Republic of Karelia for the laboratory analyses in Finland, co-operation between the plant inspection authorities of these countries is required, and therefore, one of the aims is to strengthen this co-operation. Here, we report experience and some results from the first year.

MATERIALS AND METHODS

In the first year of the project, a field experiment was set up in a grower's field in Verhovje near Petrozavodsk in the Republic of Karelia to study the effects of a fertiliser (synthetic or organic) and potato cultivars (two from Russia and two from Finland) on the occurrence of diseases. The experiment was arranged in a randomized block design with blocks nested within the fertiliser treatments. Synthetic (mineral fertiliser N:P:K=16:16:16 at 300 kg/ha and potassium fertiliser KO 50 % at 278 kg/ha) or organic fertiliser (a mixture of peat and composted cow manure at 37 t/ha) was broadcasted on the experimental area and the area was cultivated before planting. Two cultivars, Aurora and Ladoški, produced in Russia as grade Elite (EC2/EC3) and two cultivars, Saturna and Fambo, produced in Finland as grade E2 (EC2) were used. All treatments had four replications. Plot size was forty meters. The experimental area was planted, looked after and harvested according to the normal local practice by the potato farmer including four fungicide treatments against late blight (*Phytopthora infestans*). The development of the crop and disease symptoms was monitored during the growing season. For the severity of *Rhizoctonia* stem canker, the stem lesion index (RSI) was obtained by estimating the area on below-ground part of each stem covered by lesion on a scale from 0-3 and calculating a weighted average. At harvest the progeny tubers were assessed visually for disease symptoms, and analysed for the presence of quarantine pests and pathogens, black leg and soft rot bacteria (*Pectobacterium atrosepticum*, *P. carotovorum*, *Dickeya* spp.) using the method of Degefu *et al*. (2006), and potato viruses using DAS-ELISA.

RESULTS AND DISCUSSION

The experiment was successfully carried out and the difficulties with sample transfer from a non-EU country to an EU-country overcome. No quarantine pests or pathogens were detected either in the Russian seed potato or in the progeny tubers grown in the Republic of Karelia suggesting that there is no immediate thread of an epidemic. The main disease problems were caused by *Rhizoctonia* stem canker and black scurf, and viruses. High incidence and severity of *Rhizoctonia* stem canker was observed in all cultivars (Table 1), although only the seed potatoes of Russian cultivars Aurora and Ladoški were infested by black scurf. This suggests that infested soil was also a source of an infection. Black scurf was present on 60 % of the progeny tubers of the Finnish cultivars and 80 % of those of the Russian cultivars. However, the cultivars Ladoški and Fambo had the highest proportion of tubers with heavy infestation (Table 1). The proportion of tubers with heavy infestation of black scurf was increased with the use of synthetic fertiliser compared to the use of organic fertiliser. This is probably due to rapid development of late blight on the crop fertilized using synthetic fertiliser and consequent early haulm destruction. The increased period between the haulm destruction and the harvest of the tubers is known to enhance the formation of the black scurf on tubers (Dijst 1985).

Table 1. The effect of cultivar and fertiliser on Rhizoctonia stem canker and black scurf and yield.

		RSI	Stem number	Percentage of tubers with Black scurf	Severe black scurf	Yield t/ha
Cultivar						
	Aurora	1.56	2.85	80.6	7.5	17.9
	Ladoški	1.58	2.06	90.7	15.6	17.1
	Saturna	1.08	4.50	62.6	2.8	21.5
	Fambo	1.54	3.38	68.1	11.4	30.5
	F -test	*	*	*	*	*
Feriliser						
	Synthetic	1.49	3.28	71.6	13.5	20.6
	Organic	1.39	3.11	79.4	5.1	22.9
	F -test	ns	ns	ns	*	ns
Interaction	cultivar x fertiliser					
	F -test	*	ns	ns	ns	ns

* p<0.05; ns=not significant; RSI=stem lesion index

Viral symptoms were observed during the growing season especially on the foliage of cultivars Aurora and Ladoški (Table 2). The percentage infection by potato virus Y determined by ELISA test on leaf samples correlated positively with the symptoms observed. Progeny tubers of all cultivars were infected by different viruses (Table 2). Cultivar Aurora was severely infected with potato virus M, whereas cultivars Ladoški and Fambo were infected with several viruses. Both in seed potatoes and progeny tubers a latent infection of black leg and soft rot caused by *P. carotovorum* was detected.

Table 2. Incidence of viral symptoms and infection of potato viruses in leaf and tuber samples detected by ELISA.

Cultivar	Percentage of plants: Showing viral symptoms	Infected with PVY	Infection of progeny tubers with PVY	PVA	PVS	PVM	PVX
Aurora	2.3	7.5	-	-	-	+	-
Ladoški	11.7	24.2	+	-	+	+	+
Saturna	0.4	0.0	+	-	+	-	-
Fambo	1.9	0.8	+	-	+	+	+

+ infected, - not infected

In the first year, *Rhizoctonia* stem canker and black scurf and virus diseases were the most important diseases affecting the potato production in the Republic of Karelia. *Rhizoctonia* was transmitted by the infested seed and soil. Both diseases can be managed using disease free seed material together with improved cultivation methods. In the following years, different control strategies for these diseases will be tested.

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CHANGES IN POTATO PRODUCTION AND NUTRITION IN SLOVENIA

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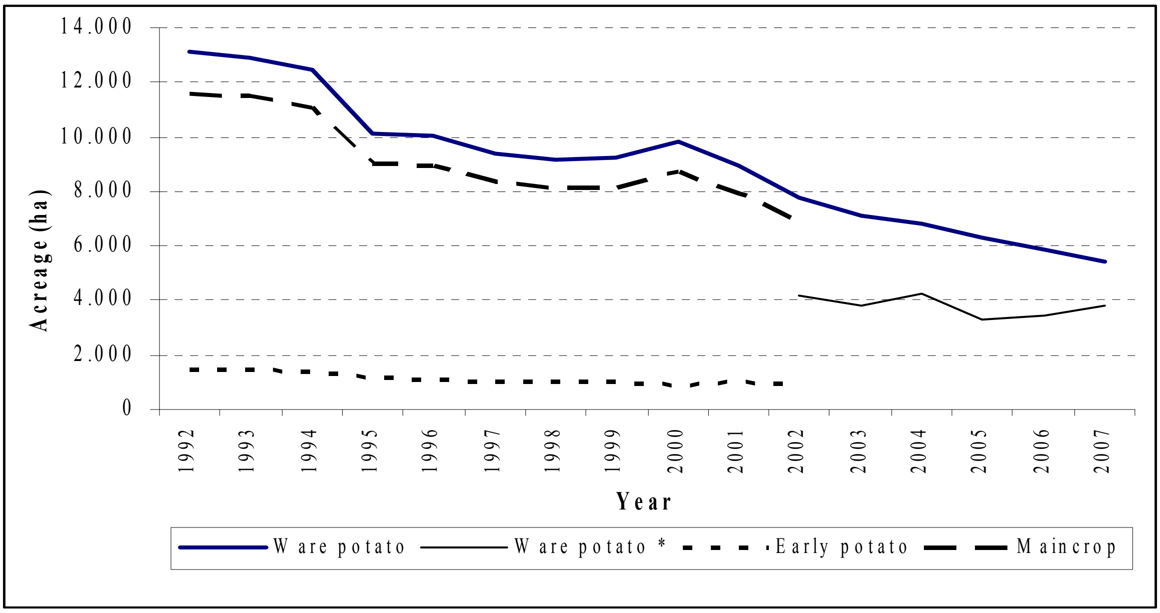
Potato used to be one of the major crops in Slovenian agriculture. It was estimated that it covered over 30.000 ha of fields all over Slovenia in early eighties, which presented over 10% of all field acreage. Yields were rather low because of less intensive growing technology and because of the use of older Slovenian varieties with low yield potential. Slovenian varieties were grown on over 80% of Slovenian fields. After an outbreak of the PVY^{NTN} virus the variety assortment changed very rapidly within a few years. The yield increased with the introduction of new varieties.

After 1990 a lot of small farmers stopped farming or at least stopped growing potatoes. On the other hand, a few of the largest farmers enlarged their production in the last few years. Some of them even purchased their own modern washing and packing facilities. The main reason was development of large merchants with supermarket chains which considerably changed the customer habits. Instead of buying potatoes for the winter on farm in autumn, they rather buy packed and washed potatoes every weekend in supermarkets. People also eat less and less potatoes and cook more pasta or rice and other foodstuffs which are widely available in the conditions of open markets. This way many small farmers lost their market and stopped producing potatoes. The data of potato production in the last 15 years are presented in Figure 1. Ware potato production dropped from 13.000 ha in 1992 to 5.400 ha in 2007 according to the data of Statistical Office of the Republic of Slovenia. The reduction of acreage was observed at both the early and the main crop potatoes. The potato acreage was estimated lower in dataset from 2002 of the Agency of the Republic of Slovenia for Agricultural Markets and Rural Development. These data were gathered from the applications for subsidies and contain only the data of the farmers who applied for subsidies. We believe that there are at least 1000 ha of small potato fields and gardens which are not included in the above mentioned dataset. Therefore the data from Statistical Office are closer to the real situation. On the other hand, a small increase can be seen in the data from the Agency in the last two years. This is probably due to the increased production of larger farmers who produce for the market (Čergan et al, 2003, Dolničar & Cunder 2004).



Source: Statistical Office of the Republic of Slovenia

Figure 1: Potato production in Slovenia from 1992 to 2007

There are also differences among individual regions in the portion of fields intended for potato, in the percentage of farms growing potato and in the average potato field area per farm (Table 1). The data are rather old, but still the only available. The situation today is similar or even changed in favour of major potato growing areas such as Gorenjska or Central Slovenia. Some regions such as Podravje or Savinjska dolina used to be the major growing areas 30 years ago, but not today anymore.

Table 1: Potato production in different statistical regions in Slovenia in 2000

Statistical regions	Acreage of potato fields		Portion of potato fields	Farms which produce potatoes		Portion of farms which produce potatoes	Average on farm potato production
	Ha	%	%	Number	%	%	Ha
Pomurje	1032.26	11.73	2.40	6714	11.51	56.99	0.15
Podravje	1100.82	12.51	3.20	8104	13.89	54.87	0.14
Koroška	377.41	4.29	12.32	2035	3.49	70.15	0.19
Savinjska dolina	815.28	9.26	5.03	8848	15.16	68.65	0.09
Zasavje	56.56	0.64	12.77	598	1.02	57.12	0.09
Spodnjeposavje	511.08	5.81	5.21	4194	7.19	72.91	0.12
SE Slovenia	1082.65	12.30	7.17	8098	13.88	84.97	0.13
Central Slovenia	1469.18	16.70	9.75	6985	11.97	73.77	0.21
Gorenjska	1344.30	15.28	21.92	3336	5.72	66.30	0.40
Notranjsko-kraška	380.88	4.33	20.70	2665	4.57	90.49	0.14
Goriška	409.91	4.66	13.32	4304	7.38	65.87	0.10
Obalno-kraška	219.64	2.50	10.63	2472	4.24	67.32	0.09
Slovenia	8799.97	100.00	5.86	58353	100.00	67.60	0.15

Source: Inventory of agricultural holdings, Slovenia 2000, SORS,

Slovenian farms are small which we can see from both Table 1 and 2. The average potato farm produced only 0.4 ha of potato in Gorenjska in 2000. It has become larger, but not considerably, in the last seven years. From the Table 2 we can see that we are far behind the neighbouring Austria or the EU 15 average. The number of farms larger than 10 ha of potato fields is especially small. Their number has increased from 4 to 20 in the last ten years (Dolničar & Cunder, 2004).

Table 2: The average potato farm size in Slovenia compared to Austria and EU 15 in 1997

Country		Size classes of farms (ha of potatoes)				Sum
		< 0,5 ha	0,5 - 2 ha	2 - 10 ha	> 10 ha	
Austria	number of farms	29480	3000	3130	300	35910
	%	82.09	8.35	8.72	0.84	100
	ha	3670	3010	13730	4200	24610
	%	14.92	12.23	55.79	17.07	100
EU 15	number of farms	657050	118550	67300	31690	874560
	%	75.13	13.56	7.7	3.62	100
	ha	95080	100340	306780	743150	1245520
	%	7.48	8.06	24.63	59.67	100
Slovenia	number of farms	52472	2174	269	4	54919
	%	95.55	3.95	0.49	0.01	100
	Ha	6248	1533	782	123	8686
	%	71.73	17.65	9.01	1.41	100

Source: EUROSTAT 1997

We believe that we have reached the dew-point in the Slovenian potato production. The acreage will probably stay at the present level, but the total number of potato producing farms will still decrease. Some larger farmers will increase their potato acreage, buy new equipment and build new storages. Lack of large fields may cause problems in some areas. There are at least eight sophisticated packing facilities operating which is enough to fulfil the Slovenian needs.

The maps of potato production according to the Inventory of Agricultural Holdings in Slovenia in 2000 and detailed maps of potato fields in 2007 using Geographical Information System (GIS) with Gauss Krueger coordinates will be presented on the poster.

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EVOLUATION OF ACTUAL SITUATION AND PERSPECTIVE OF POTATO PRODUCTION IN REPUBLIC OF MOLDOVA

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INTRODUCTION

Potato is one of the important food crops in Republic of Moldova, and plays an important roll in food strategy of country. Potato growing area amounts 25000 ha which constitutes 100% private sector. About 30% from this area belong to professional producers which covered about 75-80% from intern demand the rest are small producers. Consumption per capita is around 110 kg. Climate conditions permit to grow two yields per year: spring-summer period the main and summer-autumn so-called second crop. The main direction of production is for consumption and only a small part for seed.

PAST

Potato was introduced in Republic of Moldova at the end of 18-th beginning 19-th century. In short time areas and gross output increased very quickly, in special around big towns and villages on the irrigation fields. As a result a part of high quality production had been exported to big and importance centers as Odessa, Kiev, etc. The main cultivated variety was Early rose variety – created by Vilmoren seed company. After Second World War, potato sectors suffered very much, because a strategy of specialization and concentration in agriculture in farmer S.U. According this political decision Moldova had to be specialized in fruits, grape and vegetable production. Thousands ton of potato have bin imported from others soviet republic.

Later, closely to nigh teen years some research in technology, variety breeding and seed production started.

Avery negative period in potato production comes after S.U. broken, when the tied and weak centralized system stopped to faction.

As a results potato areas increased from 44000 ha till 65000 ha in the same time average yield field down from 9 till 6 t/ha, because a lot of unknown variety and bad seed were imported from outside.

PRESENT

Starting with 1991 a new program about variety testing and seed potato production was elaborated. A large number of varieties from different origin are tested every year. Scheme of seed potato multiplication was limited till 3 – 4 years.

In the same time only certified seeds of class E or A and only of listed varieties are allowed to be imported. Parallel many professional formers used for potato production new technology and modern equipment. From the other part potato department of Maize and Sorghum Research Institute in collaboration with same agricultural projects elaborated and applied a special instruction programs for potato growers.

Thus it is put an end to the import of unknown varieties, without known origin and is being created a system of introducing new varieties and high quality seeds, with show a high yield potential, disease resistance and appreciated culinary qualities.

In the same time a very serious attention is paid to so kind of diseases as black scurf, black leg, soft rot, verticillium sp. and in special fusarium sp. with in hot season can reduce the yield till 50%.

The efforts done led to increasing yield from 9-10 t/ha till 20 – 22 t/ha. Professional farmer obtain not less then 30 t/ha. As a results potato areas were reduced from 43000 - 45000 ha till 20000 – 22000 ha. Inside country production, practically coverage all the potato demands. Moreover starting from 2002 – 2004 Moldova made the efforts to export potato in special early production and potato for summer – autumn consumption.

The value of exported potato and turnover is not so big, but has a significance importance, because place Republic of Moldova from potato importer in to Country as an exporter of potato in quiet short time (table 1). That is why our experience could be useful for others countries

Table 1 Potato export from Republic of Moldova (Reporter Republic of Moldova)

Period	Trade value $ USA	Net weight
2002	1637	18199
2004	3726	39163
2005	57340	465580
2006	172110	741774

Export has been done in different countries in special around Republic of Moldova.

Table 2 Partners export of Moldavian potato (Reporter Republic of Moldova)

Period	Partner	Trade value $ USA	Net weight
2006	Belarus	47473	278350
2006	Kazakhstan	2100	5000
2006	Romania	150	1000
2006	World	177110	741774

FUTURE

To enlarge and stabilized potato production in Republic of Moldova the future activities should be focused on:

- early and extra early potato production for export and big supermarkets;
- introducing of modern system for potato grading, washing and packeting;
- development of food processing sector (frozen french fries, chips, flaks).

Early potato to production for export, by our opinion will develop this direction of activities and uncharged market during the summer – autumn period, when main crop varieties are ready to harvest.

Special preparation of table potato before delivered to market will mode it more attractive in special for middle class of buyers.

Development of potato industry will improve the quality and assure some quarantines for stabilization of special potato production.

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BREEDING

DEVELOPING SELECTION CRITERIA FOR BREEDING ORGANIC NITROGEN-EFFICIENT POTATO (*SOLANUM TUBEROSUM*) VARIETIES

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Introduction

The growing season for organic potatoes is, compared to conventionally produced potatoes, relatively short. On the one hand it is limited by late blight (*Phytophthora infestans*) infestation and on the other hand by a low nitrogen supply.

It is generally assumed that late blight infestation is the most important yield reducing factor in organic potato cultivation in Europe (Stöppler et al., 1990; Neuhoff, 2000). In the Netherlands the canopy of the potato crop has to be destroyed at an infestation level of 7% to avoid spread of inoculum to surrounding fields (HPA regulation, 2003). In the case of an early infestation of late blight this means the primary production processes are called to a halt when the tuber bulking rate is on its top. In years with little disease pressure, nitrogen availability is probably the most important yield limiting factor.

The organic sector in the Netherlands refrains from chemical synthetic pesticides and fertilizers. The nutrient management is based on crop rotations, solid and liquid animal manures, green manures and compost (Finckh et al., 2006). The release of nitrogen from most of these fertilizers is slow and highly dependent on soil moisture and soil temperature affecting mineralization processes (van Delden, 2001). Therefore, nitrogen management in organic production systems is very complex. The supply of nitrogen from organic resources is difficult to synchronize with crop demand (Pang and Letey, 2000).

Möller et al. (2007) state that, in Germany, nitrogen availability is the most important factor limiting yields in organic potato crops. They developed a model which in total accounted for 73% of the observed variation in yield. Only 25% of the variation could be attributed to the influence of late blight. In contrast, almost half of the variation (48%) could be explained by differences in nitrogen availability. They concluded that in organic farming, yields are mainly limited by nutrient availability in spring and early summer. The organic sector strives to work in closed nutrient cycles. However, organic manure is scarce. Therefore, the nitrogen input in organic production systems is low, compared to the input in conventional production systems. The lack of adequate and stable nitrogen supply leads to agronomic uncertainties. Modern varieties have a poor plasticity in response to variation in nitrogen availability, having small root systems, and require large quantities of nitrogen to maintain vegetative growth and productivity throughout the growing season (Vos, 1997). Such varieties are not adapted to organic growing conditions. The organic farmer prefers varieties with good yield stability, good adaptation to low nitrogen input and a good recovery capacity after a period of nitrogen shortage.

Nitrogen

Nitrogen supply affects an array of physiological processes and morphological traits of the potato crop. These include (1) the rate of canopy development, (2) the rate of leaf appearance, the rate of individual leaf growth, final leaf size, and the life span of individual leaves, (3) the integral of light interception by the crop over time, (4) the rate of photosynthesis, (5) the number of lower and sympodial branches, and (6) the onset of tuberization, final tuber yield and final harvest index (Biemond & Vos, 1992: Ewing & Struik, 1992; Vos & Biemond, 1992; Vos, 1995; Vos & MacKerron, 2000). Nitrogen supply may also affect quality aspects including tuber size distribution, tuber dry matter content, protein content, nitrate content and processing quality (Van Kempen et al., 1996).

Much research has been done to assess the yield response of varieties to nitrogen supply under high levels of input. Varieties vary in their response to high nitrogen input. This variation in response is mostly associated with differences in maturity type (Van Kempen et al., 1996). However, very little quantitative information is available about genotypic differences in response to nitrogen under low levels of. Van Delden (2001) mentioned differences in the sensibility to nitrogen shortages between the varieties Junior and Agria. Also breeders and organic farmers experience large genotypic variation in the response to low levels of nitrogen. The physiological mechanisms explaining these differences and the genetic background are, however, unknown.

Most research programs on nitrogen use in potato focus on the optimization of nitrogen supply and the timing of nitrogen supply in order to reach for each individual variety the highest yield with the best quality. To the best of our knowledge, no research is done on the opposite research question: Given a low nitrogen input, what kind of variety will be able to perform well? To answer that question one has to understand the physiological mechanisms behind nitrogen use efficiency of potatoes under low nitrogen conditions.

Project

The aim of the present project (2008–2011) is to design selection criteria for high nitrogen use efficiency under low nitrogen conditions to support breeding programs for organic potato varieties.

The first step in this research is to identify morphological plant traits that are correlated with nitrogen use efficiency and good performance under low nitrogen conditions. The next step is to translate these traits into cheap, simple and easily applicable selection criteria. We also want to do research on the nitrogen plasticity of varieties. How do they perform after a period of nitrogen shortage? What is their recovery capacity when nitrogen becomes more abundant?

The project started in 2008 with a field experiment to identify morphological plant traits that are associated with high nitrogen use efficiency. Nine varieties known by experience to differ in nitrogen requirement (Agata, Leoni, Biogold, Santé, Bionica, Fontane, Terragold, Agria and Spirit) have been tested at three nitrogen levels (low, medium and high input) on an organic and on a conventional trial field in four replicates. The soil cover is assessed twice a week, whereas leaf area, tuber bulking and nitrogen accumulation in haulm and tubers are measured over time by frequent intermediate harvests. We expect to find some parameters (a to d; see Figure 1) in the soil cover curve that are correlated with nitrogen efficiency under low input conditions.

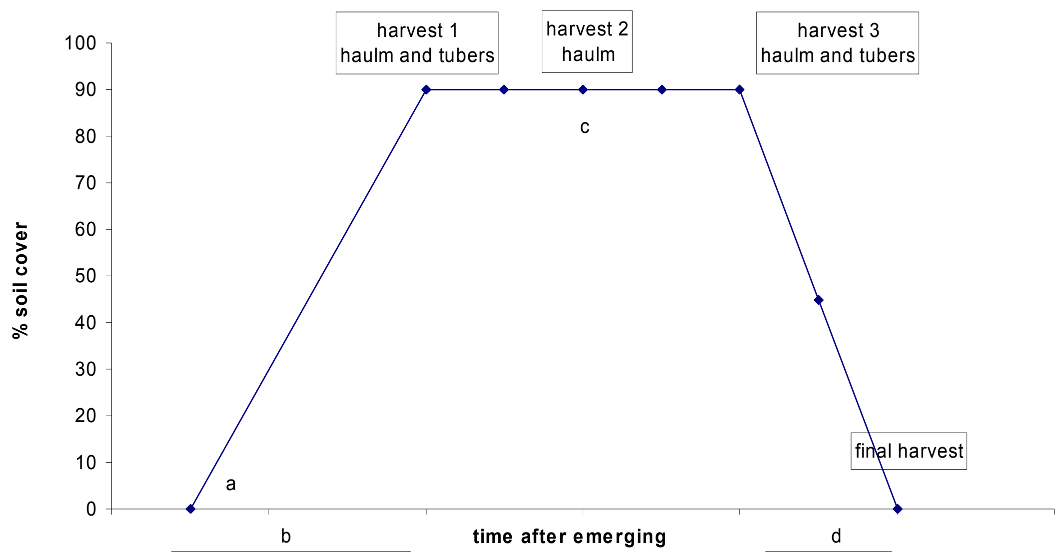

Figure 1. Soil cover curve with the parameters a, b, c and d and time of intermediate harvests (Vos & MacKerron, 2000).

a = rate of soil cover (%/day); b = time to reach maximum soil cover; c = length of period with maximum soil cover; d = time from maximum soil cover to haulm death.

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APPACALE'S BREEDING PROGRAMME AND THE APPLICATION OF NEW APPROACHES TO CLASSICAL BREEDING

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Abstract

APPACALE is a public company located in Burgos (Spain). A potato breeding programme is being developed since 1994. In 2006 two varieties were registered: NELA and JIMENA. Nela is a variety suitable for table consumption while Jimena has very good quality for crisps. Both of them have early vine maturity. Two other varieties have been evaluated in the official trials and they are expected to be released this year. The breeding program is carried out as a classical breeding programme but since 1999 inversions in applying new technologies have been made for improving and making easier the selection. Therefore, a diploid breeding programme for broadening the genetic base of our materials and molecular marker assisted selection (MAS) for accelerating the process and obtaining a higher efficiency were implemented as part of the programme. Now, apart from the normal steps of a conventional breeding programme, prebreeding at the diploid level and even somatic hybridisations by protoplasts electrofusions are being carried out. Moreover, MAS is being applied for PVY immunity conferred by *Solanum stoloniferum* and *S. tuberosum* ssp *andigena*, and for resistance to cyst nematodes (*Globodera rostochiensis* and *G. pallida*).

Keywords: potato, breeding programme, MAS, molecular markers, diploid

Introduction

Releasing a new variety can take up to 12 years so the application of new approaches for quickening the process and for the exploitation of genetic variability available in wild germplasm with high resistance to biotic and abiotic stresses is required. Thus, the first objective has been approached by MAS for an earlier characterisation of our breeding lines, while the second objective by a diploid breeding programme and somatic hybridisation. Next, the basic lines and some results of our breeding programme will be shown.

Materials and Methods

Crosses of both programmes (4x and 2x) are carried out in glasshouse in April (Ortega & Carrasco, 2005). When the different endosperm balance (EBN) of two species makes difficult the sexual hybridisation, protoplast fusion is applied (Carrasco et al., 2000). PCR markers are used for the selection of resistant clones to PVY, *G. rostochiensis* and *G. pallida* because they are very feasible to be applied in a breeding programme.

The first years of selection and seed production of our varieties are cultivated in seed production area (Valle de Valdelucio, Burgos). Starting the fourth year of field trials, the best clones are evaluated in different ware potato production areas from Spain (Castilla y León, Galicia and Andalucía)

Results

Figure 1 shows the breeding scheme of our conventional breeding programme.

	No. clones	LOCATIONS & PLOTS: SEED AREA	LOCATIONS & PLOTS: WARE PRODUCTION AREA
YEAR 0	40.000 seeds	Crossings (glasshouse)	
	25.000 seeds 120 progenies	SEEDLINGS (greenhouses)	
YEAR 1	14.000-15.000	Single plants in progenies	
YEAR 2	600-800	5-tuber plot	
YEAR 3	200-300	20-tuber plot	
YEAR 4	40-50	40-tuber plot	20 tubers plots 2 sites
YEAR 5	15-20	50x3-tuber plot Virus tested stocks	20 tubers plots 2 sites
YEAR 6	8-10	50x3-tuber plot Virus tested stocks	20 tubers plots 2 sites
YEAR 7	2-4	50x3-tuber plot Virus tested stocks	50 x 4 tubers plots 2 sites
YEAR 8	1-3	Virus tested stocks	50 x 4 tubers plots 2 sites Demonstration fields
YEAR 9		Virus tested stocks Prebasic seed lots	Official trials 6 Locations Demonstration fields
YEAR 10		Virus tested stocks Prebasic seed lots	Official trials 6 Locations Demonstration fields
		VARIETY	

Figure 1. Breeding scheme

Table 1 shows the wild species used in the diploid breeding programme and how they are employed. As APPACALE is a participant in the European project of the 6th Frame Work Programme "BIOEXPLOIT", wild species resistant to *Phytophthora infestans* (Ruiz de Galarreta et al., 1998) are mainly used. In total 5000 seeds are sown each year from this program, including 4x-seeds from backcrosses with somatic hybrids, 2x-seeds from intraespecific and interspecific crosses and 4x-seeds from sexual poliploidization by unreduced gametes.

Table 1. Wild species used in the diploid breeding programme

Wild species	Resistance	Method	Backcrosses
S. berthaultii	*Phytophthora infestans*	Sexual crosses	2
S. gourlayii	*Phytophthora infestans*	Sexual crosses	1
S. boliviense	*Phytophthora infestans*	Sexual crosses	2
S. vernei	*Phytophthora infestans*	Sexual crosses	2
S. andreanum	*Phytophthora infestans*	Sexual crosses	-
S. pinnatisectum	*Phytophthora infestans*	Protoplast fusion	2

Genes conferring resistance to PVY, *G. rostochiensis* and *G. pallida* are shown in Table 2. After studying the resistance of several varieties and breeding lines, PCR markers of these genes were applied for identifying which genotypes amplified them for a future application to their progenies. Now, we are applying markers for all of these genes.

Table 2. Pathogens and genes for MAS

Pathogen	Gene
PVY	Ry_{adg}
PVY	Ry_{sto}
G. rostochiensis	*Gro1-4*
G. pallida	QRL

At the moment, two varieties were registered in 2006 and two new materials are expected to be registered (Table 3).

Table 3. Description of the varieties and advanced lines.

Variety/clone	Parents	Destination	Earliness
NELA	Belleisle x Asun	Table potato	7-8
JIMENA	Tomensa x Hermes	Processing (crisps)	8
97P136-103	Helena x Monona	Table potato	7
99P40-2	Iroise x Fortuna	Table potato	4

Finally, Table 4 shows some of the R&D projects we are involved in.

Table 4. R&D projects

Title	Project	Funded by
Exploitation of natural plant biodiversity for the pesticide-free production of food (BIOEXPLOIT)	IP	UE – 6th Framework
Development of molecular markers for cold sweetening resistance	PROFIT	Ministry of Science and Education
Molecular marker assisted selection in potato for resistance to biotic factors	PROFIT	Ministry of Industry

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POTATO BREEDING WITHOUT POTATO VIRUS S (PVS) INFESTATION IN CZECH REPUBLIC

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In eighties improved procedures of potato maintenance breeding were adopted in most countries producing high-grade seed potato. In the framework of clonal selection large-scale ELISA detection method for complex of viruses was incorporated for field and post-harvest testing of selected plants. Due to utilisation of this method unexpected occurrence of particular viruses in basic breeding materials (e.g. PVX, or PVM) was also eliminated. (Dedic and Nohejl 1986; Dedic, 1988). Yet, insufficient performance was attained mainly owing to an uneven distribution of viruses in the plants and tubers after primary infection in the field (Dedic et al., 2001).

Later, the widespread availability and utilisation of pre-basic (nuclear stock materials), obtained from tissue-cultured plantlets, checked and maintained free of known potato pathogens and rapidly multiplied in *in vitro* conditions, changed substantially maintenance breeding of seed potatoes. Virus-free pre-basic transplants or tubers grown in protected greenhouse / nethouse are usually further propagated through open field "space isolators" and consecutive limited generation certification scheme.

One of the common potato viruses, originally widespread in most potato producing countries as a latent infection, namely potato virus S (PVS), was effectively eliminated in basic breeding materials of most cultivars utilising this intensive tissue cultures based system and subsequent laboratory checking. Nevertheless, in some cultivars / countries this virus still persists in different rate. Since 1995 also the PVS was experimentally included into evaluation of basic seed materials in CR. In the framework of research projects different control aspects were investigated e.g. preparation and annual supply of virus-free pre-basic propagation material for maintenance breeding; systematic laboratory checking of health state of pre-basic and basic grades of cultivars; evaluation of susceptibility of cultivars to PVS; different field allocation of healthy lots according to degree of PVS infestation; persistence of PVS in basic materials of imported cultivars; effect of different crop management practices etc.

The long-termed effort to eliminate PVS from basic seed potato in CR was finaly evaluated in the post-harvest laboratory ELISA test. In 1998, nearly all domestic potato cultivars (76,6 % of samples), were severely infested with PVS and only 1,4 % of samples of basic-seed (belonging to two cultivars only) were PVS-free (Dědič et al, 2002).

By 2003 severe infection with PVS had been reduced to 35,3% of samples, and 14,9% of samples were PVS-free (Dědič et al., 2004). Finally in last two years, 2006 and 2007, severe infestation with PVS was found at 2,4 % and 0,9% of samples only, and the proportion of PVS-free samples, together with multiplied imported cultivars, increased on 22,0% to 23,4% (Ptáček, Dědič, 2007). The increase of number of PVS-free or PVS low infected samples, and at the same time decrease of severely infected ones is clear evidence of successful control of virus-free breeding. Higher occurrence of PVS still persist mostly at some new breeding lines which were not yet multiplied with full utilization of virus-free breeding system developed in the course of this research. To the solution of this problem, implementation of PVS evaluation into obligatory certification criteria, for basic potato-seed in CR since 2005, contributed significantly.

Acknowledgements.
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MODERN SYSTEM FOR VARIETIES CREATION AND SEED PRODUCTION IN ROMANIA

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A good variety has to combine the yield characters, the pests and diseases resistance and the quality. To incorporate these characters in one genotype (which represent one variety) it is done sexuat hybridization between genitors with well know heredity. The constant characters are followed by a selection process in accordance with a modern scheme which took 10-12 years and where a observed more restrictive characters.

To extend the new varieties (creations) in the field it was applied a modern system, based on rapid multiplication of varieties and reduction of clonal generations to make shorter the period when the variety reach to the authorized seed producers.

System for varieties creation

The main objectives in breeding activity are:

- Increasing the yield capacity of the new varieties;
- Induction of genetic resistance to pests and enemies, especially of quarantine and also for unfavorable environment factors, first of all of drought;
- Improving the quality characters;
- Selection for varieties suitable to an ecological agricultural technique;
- Selection for varieties adapted to specialized utility.

For solving the mentioned objectives it was elaborate a short, medium and long term strategy with parameters presented in table no.1.

Table 1. The strategy for creation and promoting new potato varieties

Terms	Parameters
Short term (5 years)	Precocity
	High yield
	Resistance to: - late blight - viruses
	Yield destination: - fresh consummation - processing
	Shape of tubers: oval, round oval
Medium term (10 years)	Precocity
	Constance of the yield
	Resistance to: - late blight - early blight - viruses - cysts nematodes - hydrothermal stress
	Yield destination: - industry and processing - fresh consummation - ecological products
	Improving quality features (high protein content and less alkaloids)
Long term (15 years)	Using in dynamics the anterior parameters
	Selection of specialized varieties for a special task
	Resistance to: - aphids - Colorado beetle - Quarantine pests and enemies

Restrictive parameters: Synchytrium endobioticum, Clavibacter michiganensis

Material and methods

Like biological material there are using genitors with known heredity, represented by wild species from *Solanum* genus and varieties from *Solanum tuberosum L.* species. The genitors are selected function of principal characters taken in study.

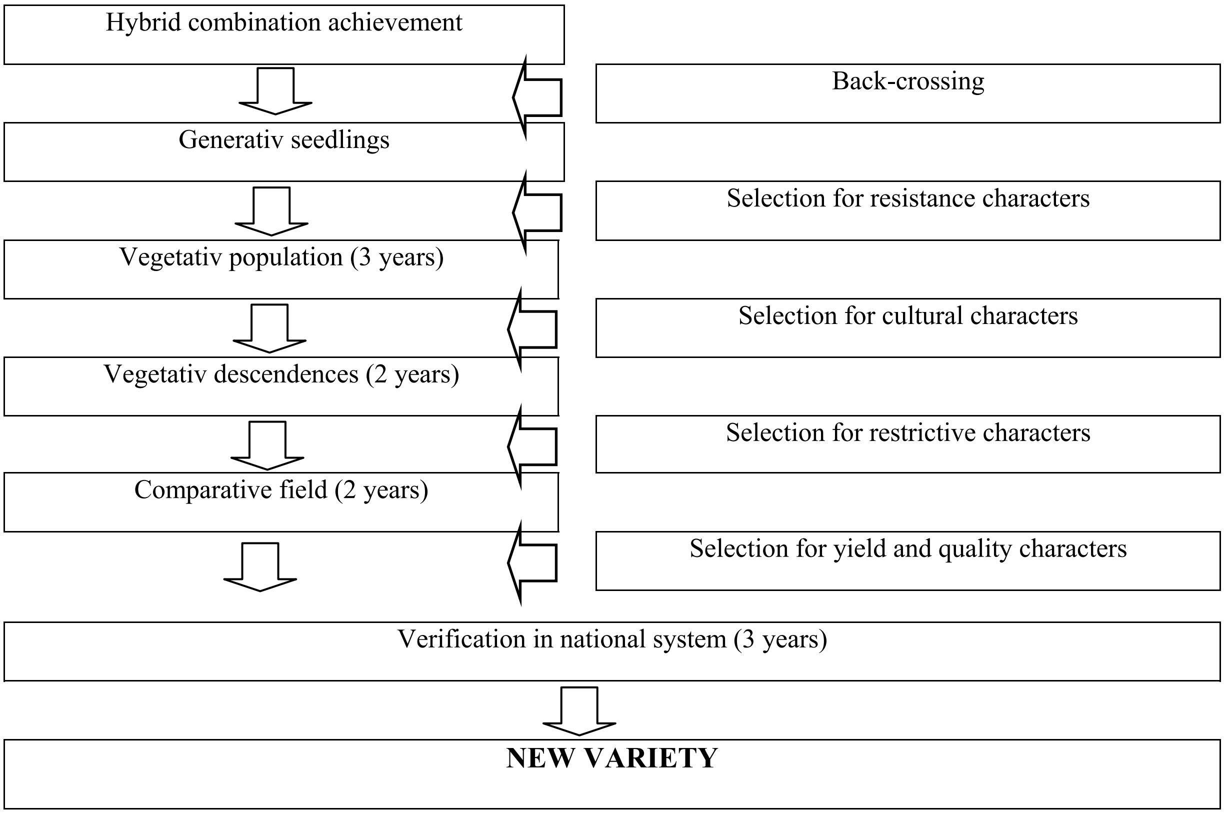
As method is used the sexuat hybridization followed by clonal individual selection.

For crossing there are taken genitors use constant character. Starting from wild species to obtain populations for selection there are made some back-crossings with varieties of the cultivated species.

Task is to improve the cultural characters maintaining the resistance.

Selection scheme is presented in figure 1.

Figure 1. Selection scheme for potato varieties



System for seed potato production

The seed production methodology must take account the reproduction mode, with essential implications:

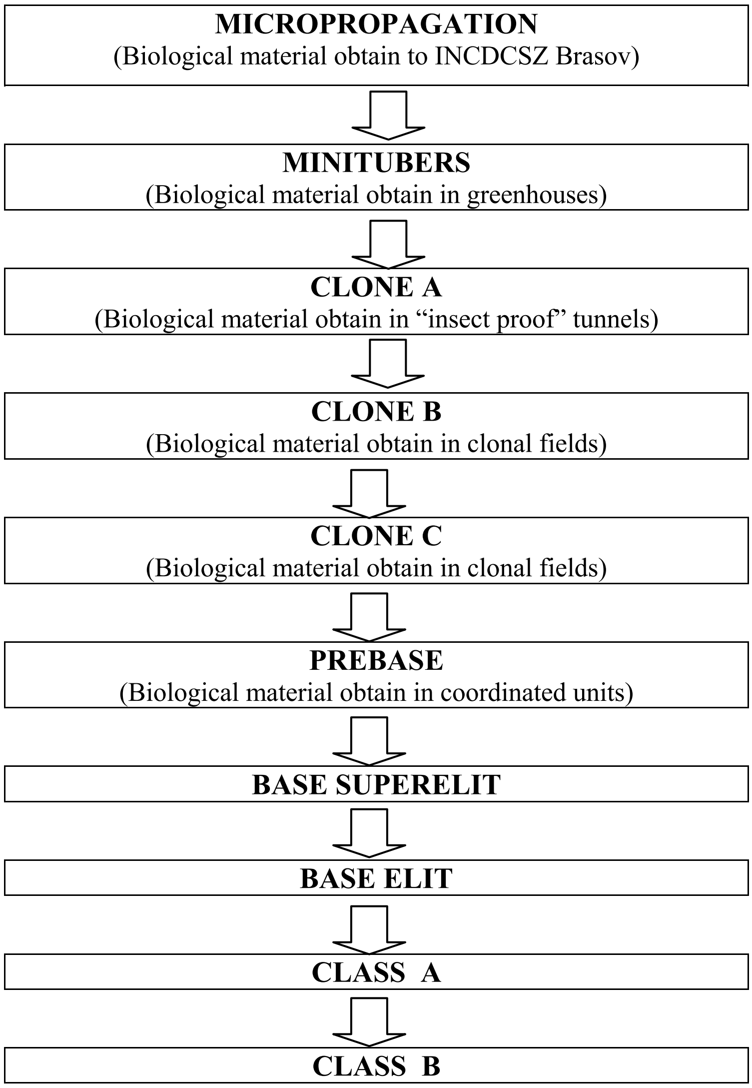
- Trough the vegetative multiplication the characters are transmitted unchanged for all the individuals coming from one descendant obtained from botanical seed. So all the plants from a variety are identical, being vegetative descendants of the same genotype;
- Trough the vegetative multiplication the planting material is expose to viruses infection, physiological stress; it is depreciate and rather reduces the yield capacity.

The consequence of degeneration is diminished by periodical replacement of planting material.

The multiple aim of utilization and the diversity of cultivating conditions impose the multiplication of a varied assortment of varieties. To obtain free viruses planted material there are used several methods. The modern one is “in vitro” multiplication starting from meristems and like cultivation system “in vitro” the most utilized for potato are: the multiplication trough microcuttings, microtubers and minitubers.

Based on producing initial material trough these methods it was elaborate the present scheme (figure 2) which is used now.

Figure 2. System for seed potato production



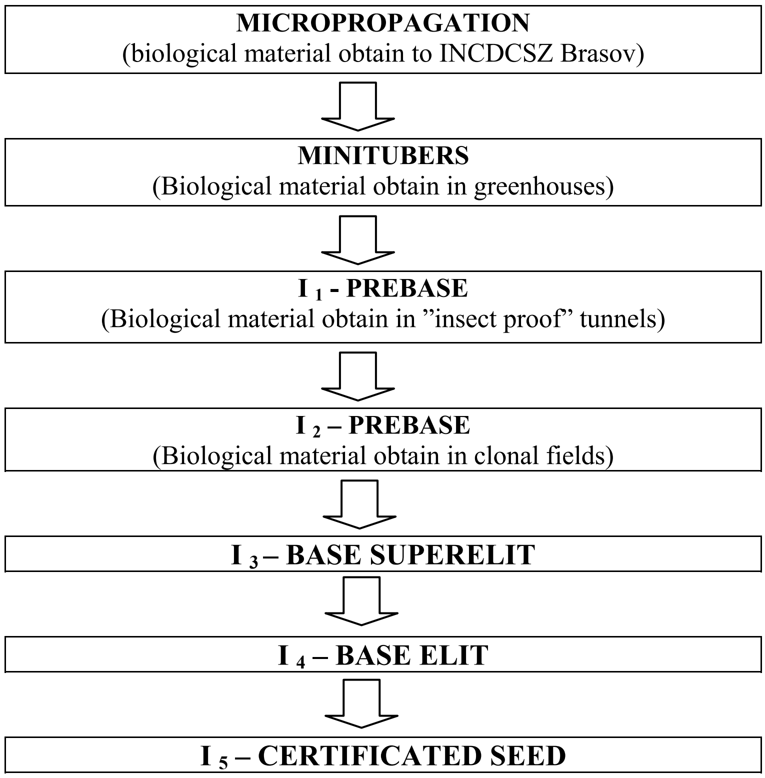
Knowing the small range of potato multiplication in normal conditions of cultivation, in average 1 to 5, depending on the variety and the great number of generations multiply vegetative in which the potato is under the influence of virotic disease, it’s impose a modernization of the actual scheme for producing seed potato in Romania.

The proposal system is presented in figure 3

The advantages of proposal system consist in:

- Increasing rate of clonal material meant to produce prebase, base and certificarted seed;
- Possibility to increase the multiplication rate in producing clonal material;
- Reduction of vegetative multiplication generations;
- Quick introduction of a new variety for cultivation.

Figure 3. The proposal system to produce seed potato



I_1 –I_5 – generations of vegetative multiplication

Conclusions

Varieties creation is a dynamic activity which aims to satisfy the demand of producers and consumers;

Omission of potato creation varieties had an unfavorable implications from many point of view in general by introduction of pests and enemies trough non adapt varieties, which can disturb the ecological environment;

If a new variety is formidable and satisfy the growers and consumers requirements must to be introduce as soon as possible in cultivation. With that end in view must be used an eficient potato seed producing system.

THE RESISTANCE VARIABILITY TO LATE BLIGHT IN HYBRID POTATO POPULATION IN ACCORDANCE WITH GENITORS AND BACKCROSS GENERATION

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INTRODUCTION

Late blight, caused by *Phytophtora infestans Mont. (de Bary)*, is one of the most significant constraints to potato production all over the world. On average yield losses due to late blight range between 28-67% in the case of sensible varieties, and between 10-20% for the resistant varieties if the protection is not sufficient (Cupsa, 1978). For avoiding the losses, the number of treatments for sensitive varieties should be around 13-15.

The objective of this study is the creation and selection of potato plants resistant to late blight, having as starting point the resistant specie *Solanum demissum Lindl.*, wich carries the R_1-R_{10} major genes, responsabile for the disease resistance.

Backcrosses with varieties which have different levels of resistance to late blight and valuable cultural characters, such as shape, size and tuber quality, were made to improve the cultural chracters of the plants. The correlation between the resistant genotype frequency in each hybrid generation and the late blight resistance of the parents used was taken into consideration.

Genetics resistance to potato late blight

Two types of resistance to late blight are distinguished regarding the mechanism of production, genetic determination and phenotypic level (Ceapoiu, Negulescu, 1983):

1. Hypersensitive resistance response, or specific, vertical resistance is conferred by single dominant genes (R genes), which are effective against specific races of *Phytophtora infestans;*

2. Field resistance, or general, horizontal resistance, which has a quantitative phenotype, is non-specific and controlled by an unknown number of genes (polygenes) and by environmental factors.

There are different types of genetic resistance or mechanisms in the host to delay or prevent attack by the fungus:

1. In specific, differential resistance the parasite induce a necrotic reaction by forming phytoalexine in the penetration area, due to plant hypersensitivity. The hypersensitive reaction is the defence of plants against pathogens that occur in an incompatible host-parasite relationship. Whenever the pathogen attacks a resistant plant, the plant reacts by the formation of hypersensitive necrosis, which prevents further spread of the pathogen. This leads, in turn, to the localization of the disease. In this type of infection the fungus does not have the opportunity to evolve. So, the resistance is absolute and in concordance with the “gene for gene” law (Robinson, 1971).

2. The uniform, horizontal resistance is conferred by a number of genetic factors in the host that slows the rate and extent of the disease’s parasitism by limiting the ability of the pathogen to become established, grow and reproduce (Robinson, 1973). The parasite is able to survive but is present at manageable levels. This type of resistance has a more general action and gives a greater stability for all races.

The genetic determinism of the late blight resistance is controlled monogenic by major genes called R-genes. The Mexican specie *Solanum demissum* Lindl. has ten R genes (R1-R10), while *Solanum stoloniferum* Schl. has only two major R gene (R6, R6+0). Other similar factors were identified in *Solanum verrucosum* Schl. The R genes are transmitted according to Mendelian law of character segregation, depending on the genetic substratum (diploid, tetraploid, hexaploid).

Material and methods

In this study observations were made concerning the behaviour of some potato populations taking in consideration especially the resistance to late blight. For this there were performed two different backcrosses using as parents hexaploid lines of *Solanum demissum Lindl*.

The combinations have the following genetic structure:

Combination 1*: Solanum demissum* x Apta)F_1 x Apta)B_1 x Schalbe)B_2 x Prosna)B_3 x Adretta)B_4 x Koretta)B_5 x Ponto)B_6

Combination 2: *Solanum demissum* x Apta)F_1 x Apta)B_1 xSchalbe)B_2 x Prosna)B_3 x Super)B_4 x Koretta)B_5 x Ponto)B_6

F_1 = interspecific hybrid *Solanum demissum* x *Solanum tuberosum*
B_{1-6} = backcross generation

These combinations are different from the point of view of the late blight resistance of the partners used as parents in backcross. The first combination (Adretta) is the resistant variety while the second one (Super) is a very sensible variety.

The verification and selection scheme of the parents resistant to blight in these populations is presented in table 1.

Verification and selection scheme of parents resistant to blight

Table 1.

Resistance compounds	Identification methods	Phase
Resistance to penetration on tissue: - minimum time to penetrate - penetration frequency	- Artificial infections on short term - Artificial infections on long term of penetration	Seedlings (%) Seedlings Note 1-9
Resistance to extension in tissue: - Minimum time to penetrate - Lesions size - Necrosis reaction	Infection of detached leaves and the incubation determination: - Note for spots: 1-9 - Presence of hypersensitivity necrosis	Clonal
Resistance to sporulation and epiphytic development: - generating time - sporulation intensity	Artificial infection of detached leaves (period in days or hours) Note 1-9	Clonal
Biochemical reaction, content of phenol and peroxidaze	Biochemical determination	Clonal

Results and discussion

Biological material was tested by artificial infection in laboratory. These tests proved that the genotypes used shown resistance to leaves attack in hybrid populations.

For inoculation it was used a sensitive variety of *Phythothora infestans (Mont.) de Bary*. The spores were collected by washing the mycelium with distillate water. The spore concentration was between 19-20000 spores/ml. The incubation was accomplished at a temperature of 4-8°C for 45 minutes.

To verify leaves resistance to late blight attack the suspension was sprayed on a very thin layer, using 100 ml suspension for 1000 plantlets.

The infection was performed after 6-8 weeks from sowing when the plants have around 4-6 leaves and they are very sensitive.

The determination of genotype frequency was realized starting with the IV^{th} backcross generation.

The genotypes frequency of the resistant combinations with different generation of backcross used in this study are presented in table 2.

Frequency of resistant genotypes to late blight in advanced backcross generations

Table 2

Combination	Frequency of resistant genotypes (%)		
	Generation 4	Generation 5	Generation 6
1	82,6	44,13	18,3
2	80,0	31,9	21,6

The conclusions that can be drawn from this study are:

By increasing the number of backcross generations, the frequency of the late blight resistant genotypes is diminished. With every backcross generation a part of the parental genetic material is lost, and with this the resistant genotypes are decreasing. For finding the resistant genotypes special selection techniques are required.

There is no correlation between the resistant genotypes frequency and the resistance of the varieties used as parents in backcross populations.

The specific and non-specific resistance does not act independently in the potato genotype. In most cases they are reciprocal.

Late blight resistance is induced by the relation between the major R genes and other minor genes involved in this process.

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DEVELOPMENT OF POTATO BREEDING RESEARCH FOR CREATION NEW POTATO VARIETIES FOR PROCESING AT STATION FOR RESEARCH AND DEVELOPMENT OF POTATO TARGU SECUIESC

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SUMMARY

Potato, from the breeding point of view, presents the following biological physiological specific features: vegetative multiplication, heterogeneity of progenitors', large plasticity, sterility, incompatible to hybridization, excessive sensibility to diseases. The first three specific features advantage the breeding process on a large scale, but the last two thus complicates the breeding activity and constitutes important problems to success.

The breeding of potato has as permanent objective the obtaining new varieties with high yield capacity, with high resistance to diseases and pest, with high quality, which have to give the satisfaction to consumers.

The creation of new potato varieties is a continue process, which has to take into account the change of ecological conditions, with increase of aggressively and pathogenity of diseases and pest, apparition of rases, stems, brotypes, pathotipes, as well as the continue increasing of consumers demands.

Keywords: *potato, new varieties, yield capacity, resistance to viruses, chips and pommes frites*

INTRODUCTION

The cultivated potato in Europe, having the origin in the South and Central America, cannot have the possibility to improve the genetically material from other species of tubers *Solanum* genre, and evaluated as a low number of genotypes[8].

The variety is a main resource for increasing the yield, without supplementary costs and energy[1].

But, each variety, as any other biological material or production equipment, has a confined time and biological degeneration with a normal wear, according to pathogens evolution, change of climatically, technical and economical condition, as well as the demand of market[6]. To give satisfaction with priority to continuously demand of consumers and producers, potato breeding is a permanent activity, of long standing, with objectives in a permanent progress, well established, which have to be satisfy by genetics' and breeders in the new creation[7].

The yield capacity, as hereditary feature, is very complex, having a great influence by climate and photoperiod[5].

The precociousness is considered recessive by genetics', having a strong correlation with yield capacity and it is conditioned by a large number of polymers genes[4].

The dry matter content, respectively starch, is a hereditary feature, which has a strong influence by geographical and agrotechnics conditions. This feature is polyfactorial, dominant. The hybride clones are heterozygote and those with high content of starch can be selected[2].

The potato breeding activity at the Research and Development Station for Potato – Targu Secuiesc, Covasna County, have been started since 1987, with very definite objectives, which give the possibility of homologation of 11 potato varieties, from which 4 varieties have breeding licenses.

In this paper is presented the methodology of obtaining and description of varieties with destination for processing: *Luiza*, *Mikel* and *Ioana* new varieties created by the Research and Development Station for Potato Targu Secuiesc.

MATERIAL AND METHODS

All varieties are obtained by sexuat hybridization followed by individual clonal selection, according to the classical scheme of potato breeding – 12 years[3].

The main steps of working method were:

Established of genitors according to physiological and technological qualities of tubers with destination for processing;

Sexuat hybridization, followed by all steps: seedlings, vegetative populations, descendants, comparative crops for completion (3 years in the network of research units and 3 years in the network of National Institute for testing and Registration of Varieties / ISTIS) and selection for maintaining in the field of clonal selection on over 1000 m a.s.l. (Apa Rosie);

Homologation, obtaining license and registration in the National List of Cultivated Varieties.

All three mentioned varieties have a high yield capacity, have a starch content over 18%, are resisting to potato cyst nematodes (*Globodera rostochiensis*), and black wart (*Synchitrium endobioticum*) and viruses. The starch content and processing quality were determined in the laboratories of SCDC Targu Secuiesc, resistance to potato cyst nematodes at Fagaras Centre, resistance to black wart at Pojorata Centre Suceava and resistance to viruses at virology Laboratory (I.N.C.D.C.S.Z. Brasov).

RESULTS AND DISCUTIONS

Variety LUIZA – have been patented in 2005/00029.

Genealogy of variety Luiza: FANAL X OMEGA

Description of plant: the plant is well developed, with a medium number of stems; the flowers are big and have white colours. The tubers are oval, with no deep eyes, the skin is yellow and the flesh is yellow. The sprouts have a middle size, they have a conic shape at the beginning of development and cylinder shape later, the terminal bud have a red – violet colours, with short lateral ramifications.

Period of vegetation: Luiza variety belongs to the group of middle late varieties, with a vegetation period of 85 – 100 days.

Yielding capacity was tested at the Station for Agriculture Research Braila and it is over 52.5 tonnes/ha.

Culinary quality is very good and belongs to B class, recommended to production of chips, having a yellow colour, after frying and 7 rate on a scale from 1 to 9. The content of starch is over 20%. This variety can be used for pommes frites production.

Resistance to diseases and pest: Luiza variety is middle sensitive to late blight on leaves and tubers, very resisting to Y (PVY) and leaf roll (PLRV) viruses, resisting to potato cyst nematodes (*Globodera rostochiensis*).

ROMÂNIA

Brevet
pentru soi de plante
Nr. 00029

Variety IOANA – have been homologated in 2003 following obtain the licence.

Genealogy of variety Ioana: M.P.I. 69 X CARPATIN

Description of plant: the plant is well developed, with a medium number of stems, the flowers have a middle size and white colours with dark yellow anther. The tubers have a round shape, with yellow skin and white flesh, which is very rare and confers a high quality of chips production.

Period of vegetation: Ioana variety belongs to the group of middle late varieties, with a vegetation period of 90 – 100 days.

Yielding capacity was tested at the Station for Agriculture Research Braila and it is over 56.2

tonnes/ha.

Culinary quality is good and belongs to B class, suitable for chips production, the colours of chips after frying is white, obtaining 9 rates on scale from 1 to 9. The content of starch is over 19%.

Resistance to diseases and pest: Ioana variety is resisting to potato cyst nematodes (*Globodera rostochiensis*), middle resistance to late blight on leaves and tubers, resisting to viruses Y.

Variety MIKEL – have been homologated in 2003 following obtain the licence.

Genealogy of variety Mikel: DESIREE X ROESLAU

Description of plant: the plant is vigorous with a large number of stems and belongs to foliage type. The leaves have a medium size with light – green colour. The flowers have a medium size, having a violet colour with white points. The tubers have a short oval shape with shallow eyes. The colour of skin is red and the colour of flesh is cream. The sprouts have a conic shape with middle size and red – violet colours on the base of sprouts. The bud of sprout on light is half open and porosity is dense to very dense.

Period of vegetation: Mikel variety belongs to the group of late varieties, with a vegetation period of over 110 days.

Yielding capacity was tested at the Station for Agriculture Research Braila and it is over 67.0 tonnes/ha.

Culinary quality is good and belongs to B class. *Mikel* variety is very suitable for pommes frites production. The content of starch is over 19%.

Resistance to diseases and pest: Mikel variety is resisting to late blight on leaves and tubers, is resisting to leave roll virus (PLRV) and tolerant to virus (PVY). It is resisting to potato cyst nematodes (*Globodera rostochiensis*), and black wart (*Synchitrium endobioticum*).

 

CONCLUSIONS

The potato varieties LUIZA, IOANA and MIKEL have a good capacity of yield, are very well adapted to soil and climate condition of Romania on the base of testing activity on the network of the National Institute for Testing and Registration of Varieties (ISTIS) before homologation.

Thanks to the high content of starch and good culinary and technological qualities, all varieties are suitable to production of chips and pommes frites.

The high resistance to viruses Y (PVY) and leaf roll (PLRV) permits the multiplication of seed potato a longer time and obtaining a more profitable yield.

The utilization of complex fertilizer (15:15:15) is efficient till $N_{150,}$ P_{150}, K_{150} level, when is possible to obtain maximum clear profit.

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„CLAUDIU" NEW POTATO ADVANCED CULTIVAR

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„Claudiu" advanced cultivar was created at the Agricultural and Development Station of Suceava.

The improving of the cultivars sets with this new genotype, represents one of the most efficient ways for increase of the yield productivity, quality and stability, and which less suffer because of less favorable biotic and biotic factors.

"Claudiu" is a semi earlier cultivar, having a high yielding potential which is associated with some superior agronomic traits

The potato cultivar "Claudiu" was created through sexuat hybridizing followed by individual clonally selection. The cultivar "Claudiu" was selected from one hybrid population which has as parental forms the cultivars Pentland J and Corona. The analyzing and testing processes were accomplished during 12 years, according by breeding classical scheme. Immediately, after germinating, the cultivar *„Claudiu"*, is remarked through a rapidly and uniform growing rhythm.

Morphological traits. The potato shrub is well developed and is formed by 5-9 thick stems, with erect - semi erect habit, edged, on green color with strong anthocianic pigmentations. The tubers shape is long oval, regularly, with superficial eyes, and the skin is plane. The suber color is red and of the pulp is light yellow.

Physiological traits. "Claudiu" is a semi earlier – semi later cultivar with a vegetation period, between 85 and 95 days. Referring to the resistance of the viruses diseases attack the cultivar "Claudiu" is very resistant to the Y virus (PUY) and middle resistant to the leaf roll of potato (PLRV), framing from the third class of the viruses degenerating. (table 1).

Concerning the resistance to manna *(Phytophthora infestans),* the cultivar *„Claudiu"* is middle resistant both to manna on leaf and tubers.. Also, the cultivar is resistant to black scabies (*Synchytrium endobioticum*) and tolerant to the common scabies (*Streptomyces scabies).*

Table 1.The resistance to the Y virus (PUY) and to leaf rolls of potato (PLRV)

Cultivar	Virus Y(PUY)		**Lef rolls of potato** (PLRV)	
	middle note	Qualifying	middle note	Qualifying
Claudiu	9,0	High resistance	7,5	Moderate resistance
Kondor	3,2	Very sensitive	6,8	Moderate resistance
Desirée	8,0	High resistance	2,0	Very sensitive

Quality. Referring to quality traits, the tubers of the cultivar "Claudiu" are framed in the coking class A/B, with starch content on 11,8%.

Yielding Capacity. The cultivar "Claudiu", in time and space, accomplished the high and stabile yield. In condition of the Braila big Island, a yield of 86 t/ha was obtained.

The cultivar Claudiu is framed of intensive cultivars group having a high yield potential.

The high resistance to the viruses diseases make possible the seed production without difficulties.

The cultivar "Claudiu" is recommended for favorable and very favorable potato crop areas, and in the less favorable are is recommended only in the irrigated conditions.

THE USE OF DISEASE PROGRESS CURVE COMPONENTS TO ASSESS POTATO LATE BLIGHT RESISTANCE TYPE IN SEGREGATING TETRAPLOID PROGENIES

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Late blight resistance is an important target for potato breeders. Breeding for partial resistance could be a way to increase durability of the resistance. However, breeding for such a complex trait is difficult. In this context, we have constructed a research program aiming at using molecular markers to help selection. A preliminary step of this program is the characterization of late blight resistance types of segregating genotypes.

In this paper we will focus on the use of different descriptors of resistance phenotypes in order to find 1) the best variables to characterise the resistance segregating in our progenies and 2) to have an overview of the magnitude of the year effect on these descriptors.

Three full sib progenies coming from controlled pollination of INRA resistant genotypes and susceptible varieties were obtained. These progenies were evaluated in the field each year from 2005 to 2007 under natural conditions of contamination. Three replicates of each genotypes were included in the trials in addition to parents of the progenies, differentials and standards. Bintje was used as a spreader between experimental rows. Experiments agreed most of the Eucablight standards (http://www.eucablight.org/EucaBlight.asp). Number of genotypes experimented ranged from 150 for one progeny to 280 for the other two.

Each year, each plant was evaluated 10 times along the life cycle using a visual scale of foliage destruction. Evaluations were made twice a week during peak of the epidemic. AUDPCr (relative area under the disease progress curve) Δa and Δt were calculated each year for each genotype (Andrivon et al., 2006). Δa is the difference between the slopes of the disease progress curves calculated on Bintje and on the observed genotype after a log transformation of the curves. Δt is the delay between the first day of infection on Bintje and the observed genotype.

Variance analyses were performed using different models. Correlations were calculated between results of different years. Calculations and statistical analyses were performed using SAS software and are not finished at the time of the abstract submission.

AUDPCr value is not always sufficient to describe resistance precisely. In Figure 1 we can see two different genotypes (C5 and AA7) of a segregating progeny coming from a cross between a resistant genotype INRA 89T117.10 and a susceptible variety Florette. C5 and AA7 exhibited comparable values of AUDPCr whereas the values of Δa and Δt were significantly different. C5 genotype is characterized by a negative Δa value and a Δt value close from zero. AA7 genotype is characterized by a positive Δa value and a Δt close from 10 days. Values of AUDPCr and Δt segregate in the same progeny (

Figure 2) indicating presence of R gene(s). We can also observe segregation of Δa (same figure) and curves of foliage destruction (data not shown) showing presence of partial resistance factors.

The use of AUDPCr components *i.e.* Δa and Δt in combination can help to classify resistance types present in the genotypes. Δt is a discrete variable, dependant on the delay between two series of notations (Andrivon et al., 2006). To calculate Δa we need to calculate the slope of the destruction curve after a log transformation. Each step of the calculation increases the error level and decreases the precision level of the estimator value.

Variance analysis on our data showed a strong year effect (Figure 4).

However, year effect was lower than genetic effects. This year effect was expected as the experiment was made in natural conditions of inoculation. In addition, year 2007 was characterized by a very early and rapid epidemic due to very rainy conditions in June and July. Despite this year effect, correlations between individual values of different year were mostly significant. However, the signification of the correlation level decreased especially for Δ variables which can be explained by the lower precision level on the variables as described above.

By the time of the congress, classification analysis will be performed to find the best way to characterize resistance types present in the progenies. We will be able to compare the data obtained in the three progenies and to compare the results obtained during each of the three years of experiment.

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Table 1: Resistance type depending on values calculated for Δa and Δt (from (Andrivon et al., 2006))

Δt	Δa	Resistance types
≤0	≥0	none (susceptible)
>0	≥0	Race-specific (RS)
≤0	<0	Race-non-specific (RNS)
>0	<0	RS + RNS (or RS not overcome)

Figure 1 Means of AUDPCr, Δa and Δt values calculated in year 2006 for C5 and AA7 two genotypes from a cross between Florette (susceptible variety) and INRA 89T117.10 (resistant genotype). AUDPCr values of Bintje is indicated as a control. Confidence interval is indicated

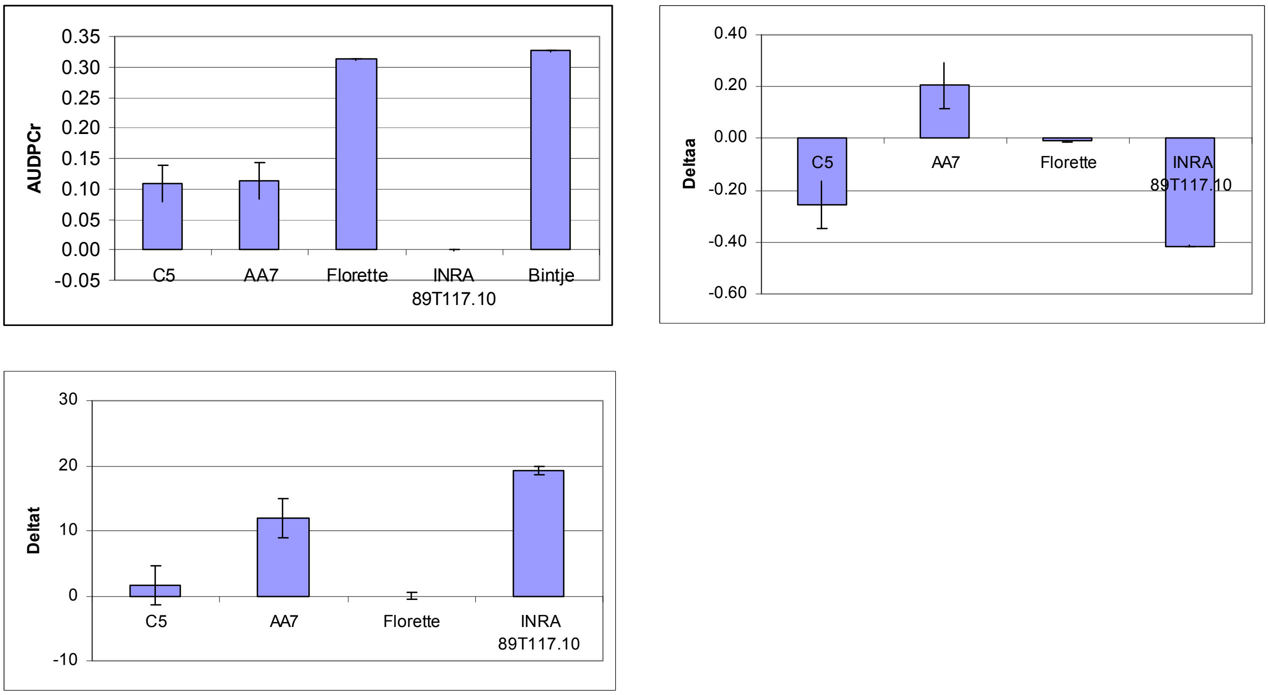


Figure 2 Histograms of AUDPCr, Δ and Δt values calculated in year 2006 for each of 216 individuals from a cross between Florette (susceptible variety) and INRA 89T117.10 (resistant genotype). X axis : number of genotypes per phenotypic class, Y axis : phenotypic classes

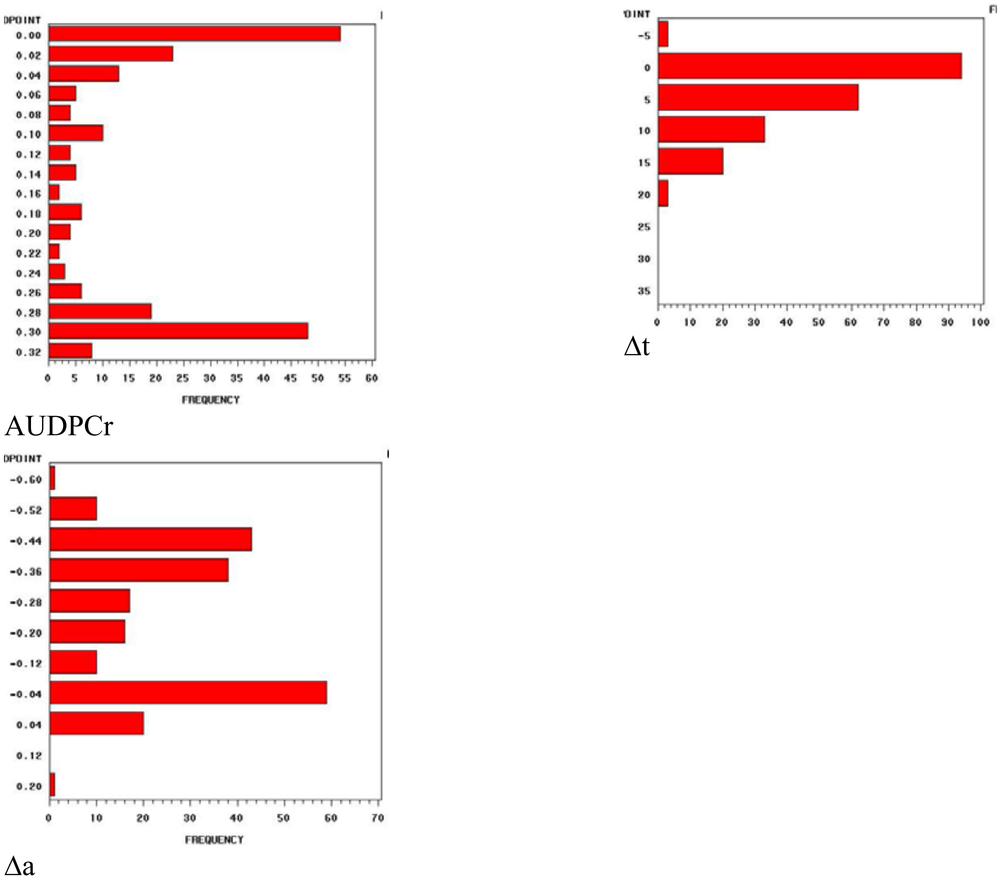
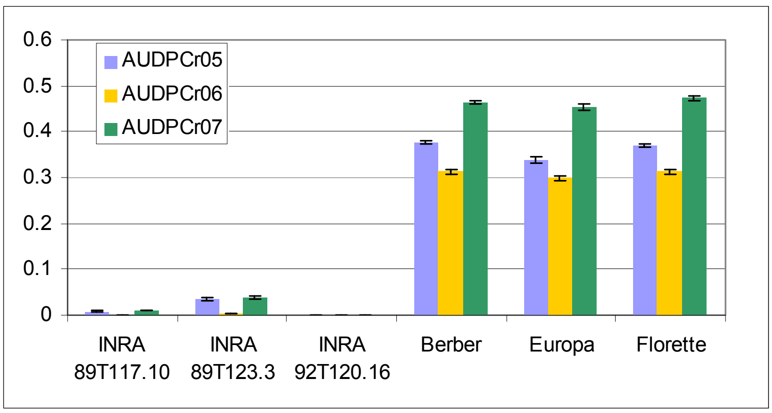


Figure 3 Ajusted means of AUDPCr values on parents from segregating populations calculated for each year of phenotypic evaluation.



GENETIC RESOURCES

THE EVALUATION OF MORPHOLOGICAL CHARACTERISTICS AND AGRONOMICAL TRAITS OF POTATO VARIETIES PRESERVED *IN VITRO*

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In EVIKA we are preserving potato varieties, breeding lines and land-races in vitro as meristem plants. The most important criterion of the preservation of genetic resources is to guarantee the genetic stability of preserved material. The aim of the project was to evaluate the characteristics of potato accessions preserved in EVIKA genebank. Descriptors for evaluation were selected from the Descriptor Lists developed by the working groups of the European Cooperative Programme for Plant Genetic Resources and promoted by the Bioversity International. In the project 53 morphological-agronomical characteristics of 119 potato accessions were evaluated.

On the basis of results the influence of long-term preservation of potato varieties in vitro on the genetic stability was clarified. The catalogue with definitions of evaluated descriptors and results was completed. The results of the study are applicable in plant breeding for further utilization of accessions.
Key words: genebank, plant genetic resources

Introduction

The preservation of plant genetic resources is the only way to guarantee their availability for the generations at present and in the future. Since it is difficult to foresee the needs of the mankind and the environmental situation in the future, it is extremely important to preserve maximum amount of species, varieties and land-races now. The greater is the diversity now the higher will be a chance to find the genes needed by users in the future.

The common preservation technique for potato varieties, breeding lines and land-races has been the preservation in field collection. In that case the material is annually planted, harvested and stored. But in field collection there is a risk for germlasm loss *via* natural disasters or infection by pests and pathogens. These risks can be avoided by introducing germplasm into tissue culture. The role of biotechnology in the preservation of potato genetic resources is inevitable and his importance is continuously increasing.

There are two major *in vitro* storage strategies for potato genetic resources: slow-growth appropriate for medium-term storage and cryopreservation in liquid nitrogen for long-term storage. In slow-growth conditions the regeneration of organs is inhibited and the sub-culturing period is prolonged. In many institutions the preservation of potato varieties with microtubers (tubers regenerated *in vitro*) are widely used (Kostrica, 1987; Kwiatowski *et al*., 1988; Espinoza *et al*., 1992; Dobranszki, 1997). To obtain microtubers *in vitro*, culture medium with different growth regulators have been used and also in different growth stage the content of medium, light and temperature conditions have been changed. The microtubers are preserved in freezer for 1...1.5 year period. The problem is that all varieties do not form microtubers *in vitro* conditions and subsequent re-growth could pose a problem.

Cryopreservation is a preservation technique of plant material at very low temperatures, commonly in liquid nitrogen at -196°C. The growth of plant tissues or organs is completely stopped. Freezing is, in principal, possible for all types of material: plantlets, seeds, shoot tips, meristems, callus, and cell suspensions, pollen etc. What kind of material to use for preservation depends on the available technique, genetic structure of species and existing genebank facilities (Engelmann, 1997). The cryopreservation - as one of the preservation method - is still in developing stage, there are still a lot of problems to study. In Agricultural Research Center Braunschweig Germany, the investigation about preservation of potato shoot tips in liquid nitrogen was done. After 3 years preservation in the re-growth experiments with 150 frozen varieties and genotypes a survival rate of approximately 82 % was obtained. At the same time the plant regeneration rate nearly 41 % was achieved. But the conclusion was – there is not enough information to make any prognoses for the length of period for plant material to be preserved in liquid nitrogen (Schäfer-Menuhr *et al.,* 1996). By using the cryopreservation technique the samples are not readily available, but need to be thawed and cultured before they can be distributed. That is one of the reasons why the additional preservation in slow-growth conditions is needed. Using the latter technique, the plants are always available and could easily be distributed.

EVIKA has the long-time responsibility for the preservation *in vitro* of potato cultivars selected in Estonia, breeding lines and local land-races with particularly valuable traits. The old varieties, which have been cultivated over long time in Estonia and some valuable Latvian and Lithuanian material, are also included.

In EVIKA the potato cultivars are preserved as meristem plants *in vitro*. All accessions are disease-free and are tested for virus infection for several times. For the disease eradication the technology created in EVIKA is used. The eradication system consists of 3 cycles: 1) selection of initial material, thermotherapy, cultivation of meristem tips and testing for virus infection; 2) re-eradication and field-testing on varietals identity, quality and disease resistance; 3) renewing the material.

The plants regenerated from meristems are initially transferred on a propagation medium containing no growth regulators. In every 3.0-3.5 months the collection is renewed by microcuttings. In every subculture 20 plants per accession are transferred to a fresh medium and the whole collection is duplicated in two storage rooms with different temperature and light regime. The preservation conditions are the following: EVIKA medium without growth hormones, average temperature 4-5°C, photoperiod 16h / 8h. All accessions, preserved *in vitro* have been preserved for the safety reason in the form of tubers, too.

In EVIKA a whole series of experiments were carried out with the aim to observe the influence of different culture medium components, growth conditions and other factors affecting the plants regeneration, productivity and the sub-culturing interval. On the bases of these experiments, the optimal preservation medium and long-term preservation conditions *in vitro* have been developed for many varieties.

Until the year 2001 the varietals identity of the preserved material has been controlled systematically but not by following the Descriptor Lists developed by the working groups of the European Cooperative Programme for Plant Genetic Resources and promoted by the Bioversity International.

The aim of the project was to evaluate the botanical characteristics and agronomical traits of Estonian and additionally Latvian and Lithuanian varieties, valuable breeding lines and land-races preserved *in vitro* genebank by using international evaluation descriptors for potato accessions; to analyze 3 years data and to complete a database.

Material and methods

In the evaluation test 31 Estonian potato varieties, 28 valuable breeding lines, 26 land-races and 10 old varieties together with 14 Latvian and 10 Lithuanian varieties (totally 119 accessions) were involved. Every year the test material initially preserved and propagated as meristem plants *in vitro* was used. The pre-growth and acclimatization of meristem plants was done in greenhouse conditions. The planting into field collection and cultivation was done by the technology for growing of potato plants created in EVIKA. 20 plants per accessions, totally 2380 plants, were planted into first year field collection.

During the vegetation period the rooting, quality of plants, flowering, disease infection and true-to-typeness were assessed. At the harvest time 40 tubers per accessions were separated for the establishment of following year field collections in 2002, 2003, 2004 (Table 1).

According to the evaluation method, the evaluation of botanical and agronomical characteristics in field collection was started with tubers and was repeated as minimum for 3 times. In the tests the evaluation and collection data on 44 botanical characteristics (tubers 11; sprouts 6; plant 27) and 9 agronomical characteristics were completed. Descriptors for 9-point evaluation scale were selected from the Descriptor Lists developed by the working groups of the European Cooperative Programme for Plant Genetic Resources and promoted by the Bioversity International (Table 2).

Table 1. Field tests

Activities		Time		
		2002	2003	2004
Agro-technical works	Forrows, fertilizer Cropcare 8-12-23, 500 kg/ha	13.05.	25.05.	10.05.
	Planting	14.05.	26.05.	11.05.
	Cultivations	30.05; 10.06.	16.06; 24.06	25.05; 09.06
	Harvest	03.09.	15.09.	14.-15.09.
Evaluations during vegetation period	Development	17.06; 02.07; 15.07.	20.06; 25.07; 30.07.	15.06; 16.07; 30.07.
	Botanical descriptors of plants	22.-25.07.	28.07; 07.08.	14.07; 06.08.
	Agronomical descriptors of plants	03.-4.09.	15.-17.09.	14.-15.09.
	Botanical descriptors of tubers	12.-13.09.	17.-19.09.	14.-15.09.
Evaluations of sprouts	Presprouting of tubers	10.04.	08.04.	06.04.
	Botanical descriptors of sprouts	08.-09.05.	05.-06.05.	03.-04-05.

Table 2. Evaluated descriptions

1	Growth habit	19	Inflorescence size	37	Flesh color
2	Foliage cover	20	Pigmentation of floral stalks	38	Flesh pigmentation
3	Plant height	21	Pigmentation of cork ring	39	Sprout shape
4	Stem thickness	22	Corolla size	40	Sprout color
5	Stem number	23	Corolla color	41	Sprout pigmentation intensity
6	Wing size	24	Corolla color intensity	42	Sprout pigmentation distribution
7	Wing shape	25	Size of white tips	43	Color of rootlets
8	Leaf size	26	Pigmentation of stigma	44	Sprout pubescence
9	Leaf pubescence	27	Berry set	45	Maturity
10	Leaf set on stem	28	Tuber shape	46	Foliage development
11	Leaf intensity of color	29	Tuber shape uniformity	47	Tuberization
12	Pigmentation of midrids	30	Eye depth	48	Number of tubers
13	Number of leaflets	31	Heel end	49	Tuber size
14	Terminal leaflet shape	32	Skin color	50	Yield potential
15	Lateral leaflet shape	33	Skin pigmentation intensity	51	Grading
16	Junction between leaflets	34	Pattern of skin pigmentation	52	General storage ability
17	Secondary leaflets	35	Distribution of skin pigmentation	53	Dormancy
18	Degree of flowering	36	Skin texture		

Results and discussion

In the project the botanical characteristics and agronomical traits of 119 potato accessions preserved *in vitro* genebank were evaluated according to the Descriptor Lists developed by the working groups of the European Cooperative Programme for Plant Genetic Resources and promoted by the Bioversity International. The evaluation was accomplished three times in field collections. The summarized data of three years' observations were analyzed and the adequate database was created. Also the catalogue with definitions of evaluated descriptors and characters references per accessions was assembled.

No deviations in morphological or agronomical characteristics of genotype were observed. By the present research it can be concluded that the medium-term preservation of potato genetic resources as meristemplants *in vitro* does not influence the genetic stability of genotypes.

The results provided valuable information about the influence of long-term preservation of meristem plants *in vitro* on the genetic stability of potato varieties. Up to present there were no similar results and the evaluation of the usability of different biotechnological methods in preservation of potato genetic resources are still going on internationally. What kind of preservation methods to use for preservation of potato genetic material, is not commonly decided yet.

The collected data give new opportunities for other institutions and breeders to use the genetic resources preserved in our *in vitro* genebank. The data also enable to use the evaluated material for the utilization and diversifying food production and agricultural environment. The materials preserved *in vitro* could be used as initial material for breeding, research, for propagation of disease-free material for seed production and for establishment of field collections.

Conclusions

Despite of the success of preservation technologies, there are still currently unsolved problems connected with preservation of potato genetic resources *in vitro* as a meristem plants, microtubers or by cryopreservation technique. Each situation needs to be matched by the best combination of conservation options, each option offering advantages and offsetting disadvantages of other methods. The methods chosen should be carefully considered, taking account of feasibility, practicality, economy and security. It is extremely important before deciding what kind of preservation technique to use, to study the influence of preservation method on genetic stability of preserved material.

Acknowledgement

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THE EXPLOITATION OF *SOLANUM CARDIOPHYLLUM* LINDL AS SOURCE OF RESISTANCE TO *POTATO VIRUS Y* AND LATE BLIGHT

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The transfer of genes from wild species belonging to section *Petota*, superseries *Stellata* to the potato gene pool is frequently prevented by reproductive isolating mechanisms and sexual barriers. Of particular interest to breeders are the resistance genes in populations of 13 indigenous diploid species (2x, 1EBN - Endosperm Balance Number) in Mexico. Heartleaf nightshade, *Solanum cardiophyllum* Lindl is a primitive Mexican potato that grows in the warm and semi-arid highlands of central Mexico. It is herbaceous and a diploid or triploid annual plant classified in the series *Pinnatisecta,* characterized by a short flowering period, very variable morphology and the production of small edible tubers. The tubers have been used as a human food source since ancient times and contain low quantities of a few glycoalkaloids.

In a program testing wild Mexican species for resistance to late blight *S. cardiophyllum* was identified as one of the most resistant to inoculates of *Phytophthora infestans* (ZLESAK & THILL 2004). This species of *Solanum* seems to be unattractive to adult Colorado potato beetles, *Leptinotarsa decemlineata* because of its slippery leaves, but resistance evaluation tests carried out in the field have not identified resistant accessions (CHEN et al. 2003), and it is unknown whether it is resistant to *Potato virus Y* (PVY).

Evaluation of accessions of *S. cardiophyllum* for resistance to pathogens and pests were made. The accession GLKS 108 from Genebank External Branch 'North', Gross Lüsewitz, of the Leibniz Institute of Plant Genetics and Crop Plant Research, Germany was selected because it is extreme resistant to PVY when mechanically inoculated with different virus strains. Using the detached leaflet assay and infecting whole plants with *P. infestans* revealed it is very resistant to foliage blight. Of the hatching larvae of *L. decemlineata* fed on the leaves of *S. cardiophyllum* only 10% survived 9 days. Laboratory trials indicated that *S. cardiophyllum* is not a suitable host for the Colorado potato beetle.

Results of systematic analysis of the crossability of wild species with cultivated *S. tuberosum* ssp. *tuberosum* using reciprocal crosses have been published. Crosses between cultivated potato and 4-7 plant introductions of *S. cardiophyllum* were unsuccessful and no seeds were obtained (JACKSON & HANNEMAN 1999). Consequently, cell fusion was used to overcome hybridization barriers and introgress multiple genes for resistance from wild species into cultivated potato.

Using mesophyll protoplast electrofusion between the wild species *S. cardiophyllum*, and two tetraploid commercial potato cvs., more than 130 somatic hybrids were produced (THIEME et al. 2004).

Table 1: Results of assessing selected somatic hybrids (H) of the fusion combination S. cardiophyllum (cph) + cv. Agave (A) and cv. Delikat (D) for resistance to PVY after mechanical inoculation in a greenhouse using virus strains (of isolates): PVY^N (CH605) – N , PVY^O (205) – O , PVY^C (Q3) – C, PVY^{NTN} (Linda) – NTN, PVY^NW (Wilga O) – W, PVY^N (Amigo-N150/1) – N*, and growing in the field using virus strain PVY^N (Amigo-N150/1) – N* - according to the excised-bud-assay, resistance to foliage blight using the detached leaflet assay with scores from 1 (susceptible) - 9 (resistant) and evaluation of morphology (nt – not tested, mix – mixoploid)

Genotype	Plo-idy	Number of plants tested/infected							Foliage blight Score ±SD	Morphology: habit, type, incidence of flowering: (+), +, ++ intensity of flowering, - no flowers
		Greenhouse						Field		
		N	**O**	**C**	**NTN**	**W**	**N***	**N***		
cv. Agave	4x	5/5	5/5	5/1	5/5	5/5	20/5	87/72	4.9 ± 0.3	Potato, tuberosum, (+)
cph	2x	5/0	5/0	5/0	5/0	5/0	20/0	14/0	8.9 ± 0.3	Wild species, +
HA 1183/1	8x	5/0	5/0	5/0	5/0	5/0	20/0	32/0	5.0 ± 2.6	Tuberosum, -
HA 1154/2	6x	5/0	5/0	5/0	5/0	5/0	20/0	12/0	5.2 ± 1.5	Poor, deformed leaves, -
HA 1155/1	6x	5/0	5/0	5/0	5/0	5/0	19/0	4/0	6.1 ± 1.4	Poor, deformed leaves, -
HA 1156/1	6x	5/0	5/1	5/0	5/2	5/2	19/0	11/0	7.3 ± 1.0	Tuberosum, (+)
HA 1164/2	6x	5/0	5/0	5/0	5/0	5/0	20/0	25/0	4.7 ± 0.7	Tuberosum, -
HA 1164/7	6x	5/0	5/0	5/0	5/0	5/0	20/0	nt	7.6 ± 1.9	Tuberosum, -
HA 1171/1	6x	5/0	5/0	5/0	5/0	5/0	20/0	19/10	nt	Poor, -
HA 1185/6	6x	5/0	5/0	5/0	5/0	5/0	20/0	10/0	7.9 ± 1.5	Tuberosum, -
HA 1186/3	6x	5/0	5/0	5/0	5/0	5/0	20/0	27/1	8.2 ± 1.1	Tuberosum, +
cv. Delikat	4x	5/5	5/5	5/5	5/5	5/4	20/20	47/36	5.3 ± 1.0	Potato, tuberosum, +
cph	2x	5/0	5/0	5/0	5/0	5/0	20/0	5/0	9.0 ± 0.0	Wild species, +
HD 1254/5	mix	nt	nt	nt	nt	nt	20/13	nt	6.9 ± 1.8	Very poor, deformed leaves
HD 1260/2	mix	5/0	5/0	5/5	5/0	5/0	20/0	4/0	7.1 ± 0.9	Tuberosum, -
HD 1263/2	mix	5/0	5/0	5/0	5/0	5/0	20/0	28/0	7.0 ± 1.5	Tuberosum, +
HD 1255/2	8x	5/0	5/0	5/0	5/0	5/0	20/0	6/0	7.3 ± 2.1	Tuberosum, large leaves, ++
HD 1262/15	6x	5/0	5/0	5/0	5/0	5/0	20/0	nt	8.3 ± 0.5	Tuberosum, bushy, small leaves ++
HD 1263/7	6x	5/0	5/0	5/0	5/0	5/0	20/0	3/0	4.6 ± 2.4	Tuberosum, strong, ++
HD 1263/8	6x	5/0	5/0	5/0	5/0	5/0	20/0	5/0	8.1 ± 0.8	Tuberosum, -
HD 1263/10	6x	5/0	5/0	5/0	5/0	5/0	20/0	12/0	4.8 ± 1.6	Tuberosum, (+)
HD 1263/13	6x	5/0	5/0	5/0	5/0	5/0	20/0	nt	8.7 ± 0.5	Tuberosum, (+)
HD 1264/2	6x	5/0	5/0	5/0	5/0	5/0	20/0	nt	8.2 ± 1.0	Tuberosum, -

The morphology, ploidy, yield and resistance to late blight and PVY of these somatic hybrids were characterized. Hybrids displayed a wide range of variation with respect to morphology similar to that of the 28 hybrids identified by SHI et al. 2006. The majority of somatic hybrids transplanted in a greenhouse were vigorous and similar to *S. tuberosum*, with an erect growth habit, large, long-oval leaflets and large white flowers and unlike *S. cardiophyllum* in not being prostrate and having small heart-shaped leaves (Table 1). Some hybrids had distorted or deformed leaflets and showed poor plant growth (Table 1). Other hybrids had a bushy habit and many small flowers like the wild parent.

The majority of the somatic hybrids of both genotype combinations showed no symptoms of virus infection after mechanical inoculation in green-house trials or when grown in the field (Table 1). The detached leaflet assay indicated that the resistance to foliage blight of these hybrids varied from susceptible to resistant (Table 1).

Backcrosses of the somatic hybrids using *S. tuberosum* as the pollen parent were generally unsuccessful. Nevertheless, two of the hybrids developed 2 small berries that were harvested and the seeds cultivated *in vitro*. Fruits with seeds were more plentiful in the field. Pollen viability of the somatic hybrids was lower than that of the potato parents, ranging between 11-58%.

The somatic hybrids cultivated in the field produced more tubers of a higher weight than plants of the wild species. There was great variability in tuber characteristics. Somatic hybrids of both genotype combinations had flat rough skinned tubers, whereas other hybrids produced smooth skinned tubers of variable shape.

A wide spectrum of somatic hybrids between *S. cardiophyllum* and commercial cultivars of *S. tuberosum*, were generated by protoplast fusion. The hybrids differed in ploidy level, vigour, leaf, and flower morphology and in the level of resistance to PVY and late blight. The results confirm that characterization and selection of parental clones is prerequisite for the efficient transfer of both the resistance to PVY and late blight of *S. cardiophyllum* into somatic hybrids. The possible exploitation of these hybrids for breeding for resistance in potato is discussed.

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PAPASALUD - EVALUATION OF NATIVE POTATO SPECIES FOR SUSTAINABLE AGRICULTURE

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Native potato species are tuber-bearing, cultivated *Solanum* species, that are not ordinary potatoes (*Solanum tuberosum* ssp.) nor *Solanum* wild species. They are cultivated under harsh environmental conditions were potato cannot compete! For centuries, these “native potato species” (NPS) have been locally selected by the Andean farmers in order to provide subsistence under the harsh environmental conditions of the Andes. The farmers were able to select and maintain a highly diverse germplasm with excellent organoleptic quality by cultivating native potatoes with different ploidy, maturity, level of resistance to pests, diseases and stresses in the same field. However, until now these valuable resources have not been exploited efficiently due to the geographical isolation (Huanco 1991).

Some old introductions of native potato species exist also in Europe. In Canary Islands the “papa negra” (=*S. chaucha*) and other old autochthonous cultivars are highly appreciated. Despite of its low productivity, the high market prices make them a lucrative crop. Occasionally, we can also find some *S. phureja* materials from Ecuador or Colombia such as the “Criolla” or “Yema de Huevo” which are sold at high prices in European markets.

Within the Papasalud project seven partners are evaluating the properties of NPS, improving and selecting appropriate materials and promoting their exploitation.

Details about the project, applied methodologies and current results can be found in the project web page: http://www.neiker.net/neiker/papasalud.

The project can be divided into four main parts. Within the 1st part of the project all NPS accessions and other local autochthonous cultivars have been acquired from existing germplasm banks or sampled from the fields. A dozen different *Solanum* species are involved. These accessions have been established *in vitro* and were propagated for distribution to other partners, or for producing the materials required for the different foreseen analyses and field evaluations. A common “core collection” including short and long-day adapted accessions has been defined to use in all experiments. In addition, selected materials will be propagated for distribution to farmers (see details on partner-specific plant materials on the project web page).

The 2nd part of the project considers beside a general morphological and physiological characterization of the materials, various analyses and field evaluations in order to determine the particular characteristics of NPS accessions and additional autochthonous or improved genotypes. These evaluations include analyses of potential contents in harmful substances (glycoalcaloids, nitrate accumulation, acrylamide formation), as well as the evaluation of their nutritional properties. Also resistances to various potato pests and diseases (viruses, nematodes, *Phytophthora*, others) including quarantine pathogens are evaluated. In some materials virus, *Phytophthora* and nematode resistance assays have been performed and several resistant accessions were found for all pathogens under study.

Some genotypes have combined resistances to different pathogens.

The agronomic performance is under evaluation as well as post harvest behaviour, organoleptic quality and processing aptitude of these materials by establishing field trials at different locations applying standard procedures (Bonierbale et al. 2007). In addition, tolerance levels to different abiotic stresses will be determined in specific environments.

These evaluations are completed with a molecular diversity screening of candidate genes for biotic and abiotic resistances and quality traits. We have analysed so far the distribution of alleles from several candidate genes (LRP, Porine, Patatin precursor, Ef1, others) in a set of native potato species using standard methodology (Sambrooks et al. 1989). The observed allelic variability varied considerably depending on the particular TDF under study.

In the 3rd part of the project pre-breeding with native potato species is performed in order to obtain superior progeny genotypes with combined or improved characteristics. Numerous crosses between different accessions have been already performed.

The 4th part of the project is devoted to promote the exploitation of NPS (Bonierbale et al. 2004). This includes a detailed analysis of market aptitude, production costs and market analyses and considers the dissemination and transfer of project results, methodologies and selected plant materials at different levels (to other project partners, to potato growers, breeders and the potato scientific community, as well as to farmers and farmer associations).

Acknowledgement

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SCREENING WILD POTATO RELATIVES FOR RESISTANCE TO PHYTOPHTHORA INFESTANS

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The INRA Solanum collection maintained in Ploudaniel (France, Western Brittany) is composed of about 7000 clones. A part of this collection has already been screened for Phytophthora infestans (Pi) resistance. Our objective is to achieve a complete characterization of the S. tuberosum wild relatives. The material is first screened for foliage resistance in semi-natural conditions. The most resistant clones are then challenged with 2 different Pi isolates performing leaf and stem tests in controlled conditions to evaluate various resistance components. The poster describes the tests used for screening and the results obtained until now.

NUCLEAR AND CHLOROPLAST DNA STUDIES USING PCR BASED MARKERS OF THE GENETIC VARIATION OF A SUBSET OF RUSSIAN POTATO CULTIVARS

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Introduction

Potato is the fourth important crop in the world and thousands of cultivars have been released. Russia is the largest potato producer after China and it has nearly 20% of the world's potato crop area (more than 3 million ha) presented by very diverse natural environments. The State Variety Register of 2007 includes 232 registered potato cultivars (State Commission of Russian Federation, 2007); out of those 188 (over 80%) were bred in Russian Federation, USSR and in NIS (ex-USSR Republics).

Most of these cultivars have been characterized for morphological and agronomical characters, disease and pest resistances (Simakov et al., 2005). However, genetic variation of Russian potato cultivars has not been thoroughly studied. Application of modern techniques based on analysis DNA polymorphism to study genetic variability of Russian cultivars is highly desirable. In this study we characterized the genetic variation in a subset of potato cultivars using nSSRs and cpDNA specific markers.

In a selected subset of 79 cultivars – 61 ones are presented in the State Variety Register. These 79 cultivars were selected due to their traits which are priorities in Russian breeding programs: resistance to potato viruses Y and/or X (Akrosia, Veteran, Golubizna, Krasnaja rosa, Nikulinskiy, Resurs, Effekt), resistance to cyst nematodes (Almaz, Dina, Givitsa, Zagadka, Kristall), suitable for processing industry (Begitskiy, Garant, Zdabitok, Lazar, Lakomka, Pobeda, Rossijanka, Rusalka, Sineva).

Material and Methods

79 cultivars were used in this study; most accessions came from the VIR potato collection. 59 cultivars were bred in Russian Federation, USSR and in NIS: *Akrosia , Alena , Alisa, Almaz, Bezhickiy, Borodjanskiy rosoviy, Brjanskiy delicates, Brjanskiy nadegniy, Brjanskiy ubilejniy,Buket, Varmas, Vesna, Veteran, Visa, Vjatka, Garant, Golubizna, Dina, Givitsa, Zagadka, Zdabitok, Ilinskiy, Iskra, Kemerovskiy, Krasnaja rosa, Kristall , Ladozhskiy, Lazar, Lakomka, Lider , Loshitskiy, Naroch, Nart 1, Narumka, Nezabudka, Nikulinskiy, Odisseiy, Pamjati Osipovoiy, Pobeda, Ramsaiy, Reserv, Resurs, Rossijanka, Rusalka, Sentjabr, Sineva, Skazka,Skoroplodnuiy,Snegir, Tomich, Udacha, Filatovskiy, Khibinskiy ranniy, Chajka, Chornuiy prince, Shurminskiy 2, Ubileinuiy Osetii, Effekt , Ubilei Zukova*.

20 cultivars have a foreign origin: *Agave, Agria, Albatros, Alwara, Anosta, Arkadia, Berlichingen, Delikat, Jessica, Karlena, Kondor, Lady Rosetta, Latona, Monalisa, Platina, Quarta, Redstar, Rikea, Saturna, Ucama.*

Young leaves were harvested from the field grown plants and frozen in liquid nitrogen. Total DNA was isolated from frozen leaf material using the method of Wienand & Feix (1980).

A set of 14 mapped nuclear microsatellites (nSSRs) (Table 1), that included the 4 from the Potato Genetic Identity (PGI) kit (Ghislain et al., 2004), was used in this study. Primers were labeled with fluorescent dyes and they were used to amplify DNA probes of the subset of potato cultivars. PCR conditions were as described Raker, Spooner (2002) and Ghislain et al. (2006). Fragment separation and detection were made in 6,5% denaturing polyacrylamide gels with the 4300S DNA Analysis System from Licor Corporation. SSR alleles were scored using SAGA GT Personal Edition Software (LI-COR). Detected alleles were scored as present (1) or absent (0).

Table 1. Primers used for the amplification.

N	Reference	Code	Repeat	T^o_m (°C)
		Nuclear SSRs		
1	*research.cip.cgiar.org*	STG0016	$(AGA)_8$	55°
2	*research.cip.cgiar.org*	STM5114	$(ACC)_7$	60°
3	*research.cip.cgiar.org*	STI0001	(AAT)n	60°
4	*research.cip.cgiar.org*	STI0004	(AAG)n	60°
5	*research.cip.cgiar.org*	STI0033	(AGG)n	61°
6	Ghislain et al. 2004, 2006	STM1104	$(TCT)_5$	57°
7	Ghislain et al. 2004, 2006	STM1052	$(AT)_{14}$ GT $(AT)_4$ $(GT)_6$	50°
8	Ghislain et al. 2004, 2006	STM0037	$(TC)_5$ $(AC)_6$ AA $(AC)_7$ $(AT)_4$	53°
9	*research.cip.cgiar.org*	STM5121	$(TGT)_5$	48°
10	Ghislain et al. 2004, 2006	STI0030	(ATT)n	58°
11	research.cip.cgiar.org	STM5127	(TCT)5	55°
12	research.cip.cgiar.org	STI0014	(TGG)n(AGG)n	54°
13	research.cip.cgiar.org	STG0025	(AAAC)5	56°
14	research.cip.cgiar.org	STG0001	(CT)10	58°
		Primers specific to cpDNA		
1	Lössl et al 1999	ALC_1 / ALC_3		44°
		CAPS markers used for molecular screening		
1	Bendahmane et al., 1997	CP 60/ Dde I		58°

The cytoplasmic type of potato cultivars was determined using specific for chloroplast DNA PCR primers ALC_1 / ALC_3 (Lössl et al., 2000) (Table 1) which able to detect the presence/absence of the 241-bp deletion of cpDNA (T/W type cpDNA respectively, Hosaka 1995). The PCR products were separated by electrophoresis in 1,5% agarose gels in TBE buffer with ethidium bromide staining. In addition, CAPS marker CP 60 and restriction enzyme Dde I (Bendahmane et al., 1997) (Table 1) was used for screening of potato cultivars to select genotypes that carry the *Rx1* resistance.

Results

Table 2 summarizes data about the total number of alleles at each SSR locus and the size ranges of these alleles as well as about the level of heterozygousity. High levels of polymorphism and heterozygosity were revealed within the analyzed subset. All SSRs used in this study were polymorphic in this subset. Of the 14 SSR loci analyzed, a total of 87 alleles were detected for all cultivars (Table 2). The number of alleles detected per single SSR locus across the 79 cultivars ranged from 3 in STG0025 to 10 in STI0033, with a mean of 6,2 alleles per locus.The highest level of heterozygosity was observed for STG0016, STI0030, STI0014, STI0033, STM0037 and STM1052 loci.

We were able to show that 14 nSSRs are useful in identification of individual cultivars (Fig.1); all of 79 potato cultivars could be distinguished.

Table 2. Characterization of microsatellite loci used in this study.

№	Code	Number of alleles detected	Allele size	Rare alleles	The number (%) of heterozygous genotypes			
						The number (%) of heterozygous genotypes with:		
					The number (%) of homozygous genotypes	2 heterozygous alleles	3 heterozygous alleles	4 heterozygous alleles
1	STG0001	7	126-140	0	32,1	39,3	17,9	10,7
2	STG0016	8	118-154	2	2,6	43,6	41,0	12,8
3	STG0025	3	193-201	1	17,9	82,1	0	0

4	STI0001	6	177-195	1	17,2	36,8	35,5	10,5
5	STI0004	9	66-105	4	11,4	41,8	45,5	1,3
6	STI0014	4	121-133	1	5,1	65,8	27,8	1,3
7	STI0030	8	71-107	2	2,7	54,1	29,7	13,5
8	STI0033	10	112-148	4	2,5	21,5	55,7	20,3
9	STM0037	8	69-91	2	7,9	36,8	36,8	18,5
10	STM1052	5	208-252	1	8,3	50,1	33,3	8,3
11	STM1104	5	166-178	1	34,6	62,8	2,6	0
12	STM5114	5	280-301	0	10,2	77,9	8,5	3,4
13	STM5121	4	282-291	1	15,0	67,5	15,0	2,5
14	STM5127	6	236-269	1	35,9	51,3	7,7	5,1
	Total	87		20				

Simultaneous use of nuclear SSRs and cpDNA specific markers could provide a better understanding of genetic relationship among potato cultivars and in a pedigree analysis. The amplified products of cpDNA revealed 59% of cultivars with the T type of cpDNA and 41% with the W type of cpDNA (Fig.2). Based on analyses of pedigrees it was shown that many of cultivars are characterized by genetic contribution of exotic germplasm (Kostina 1992); some of them have a wild type of cytoplasm (W type of cpDNA).

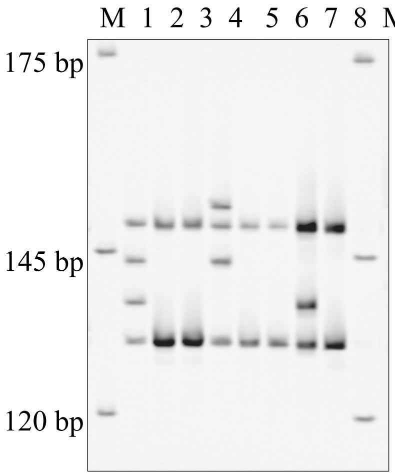


Fig. 1. Amplification products (range: 120 to 175 bp) of 8 cultivars with microsatellite STI0033:

(1) Varmas, (2) Khibinskiy ranniy, (3) Iskra,

(4) Tomich, (5) Ubileinuiy Osetii, (6) Nart 1,

(7) Resurs, (8)Vjatka.

The M label stands for molecular weight standards.

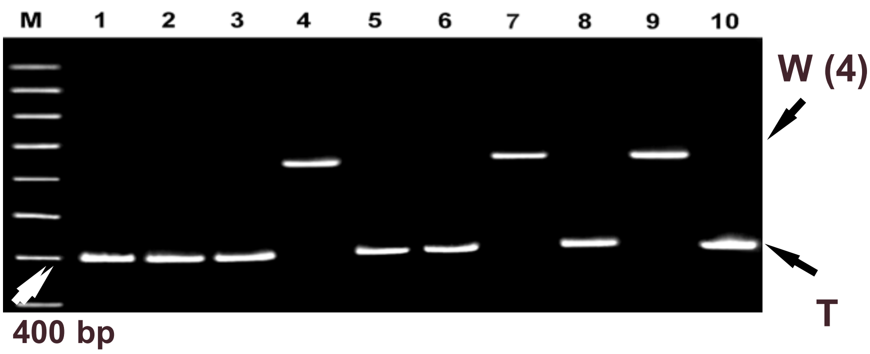


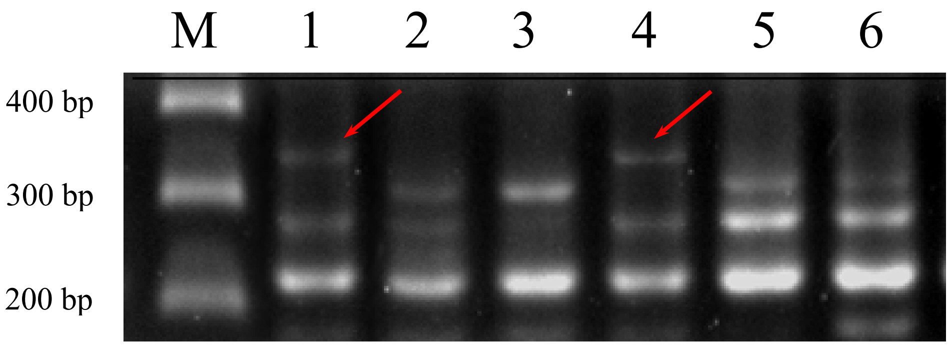
Fig. 2. Products of amplification with cpDNA specific primers ALC_1/ALC_3 in 11 cultivars (1, Ladogskiy; 2, Sentjabr; 3, Chajka; 4,Skazka; 5,Alisa; 6, Begitskiy; 7 , Garant; 8, Vjatka; 9, Borodjanski rosoviy; 10 , Zagadka). Arrows indicate different variants of revealed alleles.

M, marker of molecular weight EZ100 bp (BioRad). Electrophoresis in 1.5% agarose gel.

The genetic variation within a sample of 79 cultivars can be attributed to species (*S. tuberosum* subsp.*andigena*, *S. acaule*, *S. demissum* and some others) that were involved in the pedigrees of some cultivars (Kostina, 1992). Many of the resistance genes in modern cultivars have been introgressed both from cultivated and from wild potato species (Simko et al, 2007).

Our continuing research is application MAS (marker-assisted selection) using PCR-based markers to select cultivars with R-gene-based resistance to diseases and pests. Thus, we could identify potato cultivars that carry *Rx1* (XII) locus (Fig. 3).

Fig.3. Patterns of the CAPS marker CP 60/ Dde I amplified for DNA of cultivars: (1) Nezabudka; (2) Snegir; (3) Saturna; (4) Alisa; (5) Berlichingen; (6) Almaz. Arrow indicates marker fragment of 350 bp linked with Rx1 (XII).



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GENOMICS

APPLICATIONS OF MOLECULAR MARKERS IN POTATO BREEDING FOR LATE BLIGHT RESISTANCE

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Key words: *Phytophthora infestans,* molecular markers, potato, resistance genes

Abstract

Potato (*Solanum tuberosum* L.) late blight, caused by *Phytophthora infestans* (Mont) de Bary, is one of the most damaging diseases in potato crop (Lara Colton, 2006). Even today, the human costs of this disease can still drive modern farmers out of business. The direct monetary costs of control efforts and lost production are estimated at >$3 billion/year worldwide. Even now, more than 150 years after it was first associated with the potato late blight disease in Europe and North America, *P. infestans* remains a major problem in agriculture and recalcitrant to low-input, stable disease suppression. Advances in molecular biology and genomics promise to reveal fascinating insight into its pathogenicity and biology (Fry, 2008).

The development in recent years of DNA markers offers the possibility of developing new approaches to improving the efficiency of selection strategies. Now, the molecular markers are essential tools in potato breeding. They offer solutions to many breeding problems resulting from morphological markers. The availability of tightly linked molecular markers can be used in marker assisted selection (MAS) programs, specially for disease resistance gene. Molecular markers have potential importance in facilitating selection procedure, particularly for pyramiding two or more different genes. Breeding for disease resistance can contribute to improving quality and quantity of yield by using indirect selection through molecular markers linked to the traits of interest.

Recognizing the enormous potential of DNA markers in plant breeding, many agricultural research centers and plant breeding institutes have adopted the capacity for marker development and marker assisted selection (MAS) (Collard et al., 2005).

Introduction

Late blight is the most serious threat to potato production worldwide. *Phytophthora infestans* is a heterothallic oomycete, and it is a near-obligate hemibiotrophic pathogen under natural and agricultural conditions. The asexual cycle enables dramatically rapid population in susceptible host tissue (Fry, 2008). Late blight starts as dark, water-soaked lesions present on the leaf surface that rapidly spread over the foliage. The devastation caused by *P. infestans* was first noticed during the middle 19th century Irish potato famine, which resulted in one million deaths. Late bligh is still the most serious problem of cultivated potatoes and may cause complete tuber loss in susceptible germplasm. Current potato production practices use expensive fungicide applicationsand intensive cultural practices to control *P. infenstans* outbreaks. Due to the constant genetic shifts in *P. infenstans* population the decrease in fungicide effectiveness, this pathogen can cause significant losses in potato (Lara Colton et al., 2006).

Many agriculturally important traits such as yield, quality and some forms of disease resistance are controlled by many genes and are known as quantitative traits. The regions within genomes that contain genes associated with a particular quantitative trait are known as quantitative traits loci (QTLs). A major breakthrough in the characterization of quantitative traits that created opportunities to select for QTLs was initiated by the development of DNA (or molecular) markers in the 1980s (Collard et al., 2005).

The genetic basis of resistance to *Phytophthora infestans*

Genetically controlled disease resistance to plant pathogens can be classified as either vertical or horizontal resistance. By classical breeding both types of resistance to *Phytophthora infestans* have been introduced into potato. Vertical resistance, governed by the so-called R-genes, is single gene based. 11 dominant R-genes, all originating from *Solanum demissum* (L) have been introduced into the cultivated potato by breeding. In contrast to vertical resistance in which a single R-gene is sufficient to protect the plant completely against particular races of *Phytophthora infestans* , horizontal resistance, also called race-nonspecific resistance, is assumed to be multiple gene based. This tipe of resistance is durable and thus commercially more attractive than race-specific resistance (Pieterse et al., 1992).

Genetic markers

Genetic markers represent differences between individual organisms or species. There are three major types of genetic markers:

- *morphological markers,* which themselves are phenotypic traits or characters ;
- *biochemical markers,* which include allelic variants of enzymes called izozymes;
- *DNA (or molecular) markers,* which reveals sites of variation in DNA.

DNA markers may reveal genetic differences that can be visualized by using a technique called gel electrophoresis and straining with chemicals or detection with radioactive or colorimetric probes. The advantages and disadvantages of the most commonly used markers are presented in Table 1 (Collard et al., 2005).

Table 1. Advantages and disadvantages of most commonly-used DNA markers for QTL analysis

Molecular marker	**Codominant (C) or Dominant (D)**	**Advantages**	**Disadvantages**	**Raferences**
Restriction fragment length polymorphism (RLFP)	C	-Robust -Reliable -Transferable across population	-Time consuming, laborious and expensive -Large amounts of DNA required -Limited polymorphism	Beckmann& Soller (1986), Kokert (1994), Tanksley et al. (1989)
Random amplified polymorphic DNA (RAPD)	D	-Quick and simple -Inexpensive -Multiple loci from a single primer possible -Small amounts of DNA required	-Problems with reproducibility -Generally not transferable	Penner (1996), Welsh & McClelland (1990), Williams et al. (1990)
Simple sequence repeats (SSRs) or "microsatelites"	C	-tehnically simple -robust and reliable -transferable between populations	-large amounts of time and labour required for production of primers -usually require polyacrylamide electrophoresis	McCouch et al. (1997), Powell et al. (1996), Taramino& Tingey (1996)
Amplified fragment Length Polymorphism (AFLP)	D	-multiple loci -high levels of polymorphism generated	-large amounts of DNA required -complicated methodology	Vos et al. (1995)

Marker-assisted selection

There has been a lot of excitement about marker-assisted selection (MAS) in plant breeding over the past fifteen years. Within a few years, high-density DNA marker maps had been constructed for nearly every important crop species.

Marker-assisted selection (MAS) is a method whereby a phenotype is selected on the genotype of a marker. Generally, the steps required for the development of markers for use is MAS includes: high-resolution mapping, validation of markers and possibly marker conversion.

Selecting plants in a segregating progeny that contain appropriate combination of genes is a critical component of plant breeding. Marker assisted selection may greatly increase the efficiency and effectiveness in plant breeding compared to conventional breeding methods. Once markers that are tightly linked to genes or QTLs (quantitative trait loci) of interest have been identified, breeders may use specific DNA marker alleles as a diagnostic tool to identify plants carrying the genes or QTLs.

The advantages of MAS include:

-time saving from the substitution of complex field trials with molecular tests;

-elimination of unreliable phenotypic evaluation associated with field trials due to environmental effects;

-selection of genotypes at seedling stage;

-gene "pyramiding" or combining multiple genes simultaneously;

-avoid the transfer of undesirable or deleterious genes;

-selecting for traits with low heritability;

-testing for specific traits where phenotypic evaluation is not feasible (Collard et al., 2005).

Molecular markers have been developed for selection of several important disease resistance traits in potato. Restriction fragment length polymorphism (RFLP)and sequence-characterized amplified region (SCAR) have been identified for use in identifying potatoes containing the Ry_{adg} gene conferring resistance to *Potato virus Y*. PCR based markers were developed for resistance to potato root cyst nematode [*Globodera rostochiensis* (Woll.) Skarbilovich]. Significant efforts for genetic mapping and marker development have been made on quantitative resistance to *Phytophthora infestans.*

Lara Colton et al. (2006) demonstrated that that the high level of late blight resistance in a wild potato relative *S. bulbocastanum* Dunal subsp. *Bulbocastanum* is mainly controlled by a single resistance gene RB. Marker based selection will allow to transfer the RB gene into potato using traditional breeding methods. They had developed a polymerase chain reaction (PCR)-based molecular marker for tracking the gene RB in breeding population. This marker can be used effectively for marker-assisted selection of the RB-mediated late-blight resistance (Colton et al., 2006).

The promise of MAS in plant breeding remains, though achieving the practical benefits is clearly taking longer than most had expected. To take advantage of this powerful technologies, research teams, governmental funding agencies, and even the commercial sector will need to work together to insure that public breeders are using the best tools possible.

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COPY NUMBER AND TRANSCRIPTION LEVEL IN *SOLANUM CARDIOPHYLLUM* TRANSFORMANTS WITH DIFFERENT ORIGIN AND PLOIDY

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In order to assay the effect of regeneration events on both transgene copy number and expression level, three different genotypes of *S. cardiophyllum* were used in genetic transformation experiments. In particular we used a wild type clone (CPH1C; 2n=2x=24) and two regenerated derivatives N11B and C6B, respectively diploid (2n=2x=24) and tetraploid (2n=4x=48); *S. tuberosum cv.* Désirée (2n=4x=48) was used as control. *Agrobacterium*-mediated transformation was done according to either Millam (2004) or Karp *et al.* (1984) procedure by using a *p35S::GUS-INT* vector harboring the transgene *uidA* under a double 35S promoter. The ploidy level of transformants was checked by counting chloroplast number in stomata guard cells of epidermal strip leaves and the number of somatic chromosomes in root tips. DNA and RNA were extracted from transformed plants by DNeasy Mini kit and RNeasy Mini kit, respectively. *UidA* copy number and mRNA relative abundance was assayed by Real Time qPCR.

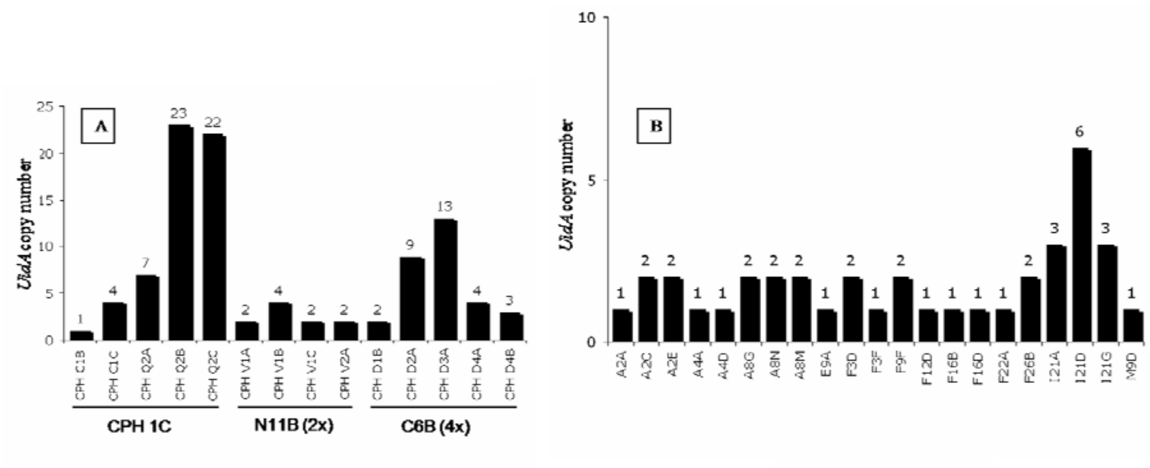

Figure 1. UidA copy number assayed by Real Time qPCR in transformants of S. cardiophyllum (A) and S. tuberosum cv. Désirée (B). Transformants of S. cardiophyllum are grouped according to the genotype they are generated from.

Transgene copy number stably integrated in transformant genomes varied significantly between genotypes. On average, *S. cardiophyllum* transformants integrated more copies of *uidA* transgene than *S. tuberosum* (respectively 5.50 *vs.* 1.65). The origin of explants affected the transgene copy number (Fig. 1). In fact, transfromants coming from the wild type CPH1C showed a significantly higher transgene copy number compared to transformats coming from N11B and C6B. Also, significant differences were observed in copy number between transformants coming from diploid and tetraploid derivatives suggesting the transgene integration being affected by the ploidy level (Fig. 1). In addition, *uidA* transcript showed significant variations between genotypes. Again, *S. cardiophyllum* transformants showed higher level of the *uidA* transcript compared to *S. tuberosum* ones. Within *S. cardiophyllum* transfromants a strong effect of the origin of explants was observed on the *uidA* transcript abundance. In fact, transformants coming from the wild type CPH1C showed a significantly higher transcript RQ when compared to transformants coming from N11B and C6B. Finally, transcript level showed to significantly increase moving from N11B to C6B transformants underling an effect of the ploidy level,

This work suggests that regeneration events may compromise the transgene integration through *Agrobacterium*-mediated transformation and limit the transcription of the transgene in further transformants. Additional studies will investigate molecular mechanisms elicited by somaclonal variations and affecting transgene expression in transformants coming from regenerated tissues.

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ASSOCIATION MAPPING IN TETRAPLOID POTATO USING SSRS AND AFLPS

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224 cultivars, including important progenitor clones, have been selected to represent the genetic diversity of the potato gene pool. These genotypes have been phenotyped for agro-morphological and quality traits in a replicated field trial. The genome has been scanned with ~1500 AFLP markers from 22 primer combinations, supplemented with ~50 SSR markers. The SSRs have been scored with methodology allowing genetic discrimination between individual allele dosages. If appropriate, null alleles have been postulated. Marker-trait associations have been analysed with a mixed model association mapping approach, where genotypic information can be used qualitatively as well as quantitatively. The qualitative approach is based on absence or presence of marker alleles, the quantitative approach is using fragment peak intensity as a measure for zygosity. Highly significant associations have been obtained, confirming previous knowledge but new QTLs have been detected as well. Correction for population structure was an essential step to remove spurious associations. In addition, we will discuss the extent of linkage disequilibrium within the tetraploid potato genome. This study illustrates the potential of association mapping as a tool for unravelling complex traits, even within an autotetraploid species like potato.

STUDY OF GENETIC VARIATION OF EUROPEAN AND AMERICAN VARIETIES OF SOLANUM TUBEROSUM L. USING SSR MARKERS

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Solanum tuberosum L. (potato) is one of the most valuable plants which belongs to Solanaceae family. Historically, *S. tuberosum* varieties have been discriminated and identified through using various morphological characteristics. However, many of these morphological traits can vary drastically with environmental conditions. On the other hand, they are seldom available all the time. Therefore, nowadays rapid and efficient methods like molecular methods are used for identification of *S. tuberosum* varieties. Microsatellites are suitable to study the genetic relationships between low level taxonomy of *S. tuberosum* varieties due to codominant, multi-allelic and abundant frequency within genome. So, in this study genetic variations of 26 European and American potato varieties have been evaluated using 6 SSR markers. After DNA extraction from fresh leaves of *S. tuberosum* plant using CTAB method and amplification of fragments by PCR, amplificated samples have been transferred on polyacrylamide gel for precise analysis. In total, 6 SSR markers produced 28 alleles with an average of 4.67 per locus. Average PIC and heterozygocity percentage have been estimated 0.64 and 73% per locus, respectively. The resulting dendrogram based on Jacquard similarity coefficient and UPGMA analysis, could distinguish all varieties but two varieties of Ayg-2 and Yucon Gold, so that most of the European varieties have been separated from American varieties and located in a distinct group. The average of similarity coefficient between European varieties was higher in comparison with American varieties, which presents more genetic variation in American varieties in comparison with European varieties. So, using more extensive studies in future and varieties with wider geographical range, American varieties can be selected as valuable genetic source in breeding programs of *S. tuberosum.* Also according to morphological traits problems in the identification of *S. tuberosum* varieties, using SSR markers can be a considerable help in identification and taxonomy of this plant.

Key Words: Microsatellite, Codominant, Genetic variation

MOLECULAR BREEDING

DIPLOID HYBRIDS BETWEEN ALLOTETRAPLOID WILD POTATO SPECIES AND DIHAPLOIDS OF *SOLANUM TUBEROSUM* L.

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Allotetraploid potato species, such as South-American species *Solanum acaule* (***acl***) (series *Acaulia*) and Mexican *S. stoloniferum (**sto**), S. fendlery* (***fnd***), *S. polytrichon* (***plt***) (series *Longipedicellata*) are the source of valuable genes of pest and disease resistance and resistance to abiotic stresses. Introgression of these genes into the gene pool of cultivated potato *S. tuberosum* (***tbr***) provides opportunity for breeders to create more effective potato cultivars [1 etc].

These species are classified as the 4x(2EBN) group of species, which is considered to be the most archaic and primitive among representatives of the *Petota* series [2] and has disomic trait inheritance. In spite of the fact that these species are tetraploids, because of their disomic nature and effective ploidy (EBN) equal to 2, they cannot crossed with tetraploid ***tbr***, which is a tetrasomic 4*x*(4EBN) species. On the other hand, these species have no prezygotic barriers and can be easily crossed with ***tbr*** dihaploids (2*x*(2EBN)) and other 2*x*(2EBN) species with triploid 3*x* (2EBN) hybrids formation [3 etc]. Such triploid hybrids 3*x*(2EBN) are viable breeding material but usually completely sterile because of significant mega- and microsporogenesis disturbances, that result in isolation of allotetraploids from diploid potato species during their evolution [2].

Different approaches were used to overcome reproductive barriers between disomic and tetrasomic tetraploid species for introgression of valuable gene pool of wild species in breeding process of potato. Some of them included complex ploidy manipulations with initial material or hybrids, others used different in vitro techniques, for example, somatic hybridization via protoplasts fusion [4-6 etc]. Many of these methods are difficult and in most cases not sufficiently resultative. In addition, breeding the valuable hybrids was hampered due to necessity of performing selection among highly polyploid (at best, tetraploid) and aneuploid material characterized by complex segregation. Much time is needed for stabilization of the ploidy on the tetraploid level of breeding material and elimination of unfavorable characteristics of the wild species.

The new method is proposed in this paper for producing hybrids between allotetrapoloid species and cultivated potato. It is based on selection of diploids among interspecific hybrids obtained by pollination of allotetroploid species with highly fertile pollen of ***tbr*** dihaploids.

Pollination of different clones of allotetraploid species with the pollen of fertile diploid ***tbr*** leads to the formation of huge amounts of berries with normal seeds (Table 1). Efficiency of hybridization (number of seeds/pollination) was mostly determined by level of fertility of female forms and was somewhat higher for *acl*. At the same time, only one ***tbr*** combination (***tbr*** 882-5×***sto*** K2534/2) was successful (4,8 seeds per pollination) from 14 combinations of reciprocal crosses (pollination of ***tbr*** dihaploids IGC-94/135.20 и 882-5 with pollen of different ***acl, sto, plt*** and ***fnd*** clones).

Table 1. Results of hybridization between allotetraploid species *S. acaule* (*acl*), *S. stoloniferum* (*sto*), *S.polytrichon (plt)*, *S fendlery (fnd)* and dihaploids of *S. tuberosum* (*tbr*)

Maternal sample	Number pollinated flowers	Berries formed	Seeds obtained	seeds per pollination
acl C46- 1[1]	2	2	204	102
acl C46-1[2]	3	2	102	34
acl C46- 3[3]	11	2	199	18.09
acl C46- 3[4]	3	1	54	18
acl C46-3[2]	2	2	262	131
acl C46-12[1]	10	4	306	76.5

sto A32-1[1]	18	6	441	24.5
sto A32-3[1]	13	2	83	6.38
sto A32-4[1]	26	9	82	3.15
sto L32-7-1[2]	3	1	57	19
sto L32-7-2[2]	10	2	87	8,7
sto K3533[2]	19	8	321	16,89
sto K3226/1[2]	29	8	93	3,21
sto K3326/2[2]	19	10	67	3,53
sto K2534/1[2]	7	0	0	0
sto K2534/2 [2]	16	4	114	7,13
sto K17152	5	2	29	5,8
plt L44-18[2]	4	3	14	3,5
plt K20086/2[2]	2	0	0	0
plt K20086/2	31	4	3	0,097
plt K20086/3[2]	7	0	0	0
plt K5682/2 [2]	23	9	641	27,87
plt K5682/3[2]	2	1	92	46
fnd L46-17[3]	15	3	76	5,07
fnd L46-17[2]	8	1	15	1,88

[1] Mixture of pollen from four clones of the dihaploid of *tbr* IGC-98/124.n (the clone IGC-98/124.n obtained from the cross Yu1.2 × (Yu1.2 × IGC-203/5.7-5), the clone Yu 1.2 was obtained from open pollination of primary dihaploid of the cultivar Yubel; the clone IGC-203/5.7-5 was obtained from self-pollination of the secondary dihaploid *tbr* LVD203/5.7 of the cultivar Polesskiy Rozovyj.

[2] The Pollinator 882-5. was obtained from the cross [(Yu1.2 × IGC-203/5.7-20) ×Yu1.2] × A15; the clone A15 was obtained from *S. chacoense*; IGC-203/5.7-20 was obtained from self-pollination of the secondary dihaploid *tbr* LVD203/5.7

[3] The Pollinator IGC-94/135.20 was obtained from the cross Yu1.2 × LVD 203/5.7;

[4] Mixture of pollen of *tbr* IGC-203/5.7; IGC-94/135.20; 895-40; 885-11dihaploids. The clones 895-40 and 885-11 were obtained from the cross [(IGC-94/135.20) × Yu1.2] × Yu1.2.

Viability of seeds and plantlets obtained from interploid crosses was lower than for diploid interspecies potato hybrids with ***tbr*** as one of the parents (according to our observations). Most hybrid plantlets did not formed tubers, and those, which formed tubers, had late formation of small tubers and multiple branching stolons.

Backcross of the part of F_1 hybrids with mixture of pollen from ***tbr*** dihaploids resulted in production of some berries with viable seeds (Table. 2). It was not possible to obtain berries by pollination the same hybrids with mixture of pollen from tetraploid varieties.

Cytological study of ploidy of some hybrids forming seeds after pollination with pollen of diploid ***tbr*** revealed their diploid level. Meiosis in these forms was normal with regular segregation of chromosomes in MII 12+12, except distortions in some meiocytes.

Table 2. Results of backcrossing F1 hybrids between allotetraploid species and *S. tuberosum* (*tbr*) dihaploids with *S. tuberosum* (*tbr*) dihaploids

Hybrid combination F1	Number of pollinated clones	Frequency of F1 hybrids able to hybridize with *tbr* dihaploids, number and (%)	Results of backcrossing of individual F1 hybrids with *tbr* dihaploids		
			F1 hybrids	Seeds obtained (number)	seeds per pollination (number)
acl ×tbr					
IGC-00/123.n	13	2 (15,38)	IGC-00/123.1	1485	59,4
			IGC-00/123.2	668	19,09
IGC-00/130.n	8	1 (12,5)	IGC-00/130.10	25	1,67
IGC-01/151.n	4	0 (0)	--	--	--
IGC-02/199.n	5	1 (20,0)	IGC-02/199.2	272	90,67
Total	30	4 (13,33)		2450	
sto ×tbr					
IGC-01/19.n	28	6 (21,43)	IGC-01/19.1	3522	234,8

			IGC-01/19.2	707	47,13
			IGC-01/19.4	862	66,31
			IGC-01/19.10	746	31,4
			IGC-01/19.12	287	15,98
			IGC-01/19.13	320	80,0
IGC-01/20.n	18	3 (16,17)	IGC-01/20.1	384	25,6
			IGC-01/20.3	365	45,62
			IGC-01/20.4	60	30,0
IGC-01/21.n	6	2 (33,33)	IGC-01/21.7	3	0,16
			IGC-01/21.16	4	0,17
Total	52	11 (21,15)		7260	
plt*×*tbr					
IGC-01/24.n	8	0 (0)	--	--	--
IGC-01/25.n	20	4 (20,0)	IGC-01/25.2	> 500*	
			IGC-01/25.6	638	17,72
			IGC-01/25.11	> 500*	
			IGC-01/25.15	> 500*	
Total	28	4 (14,29)		> 2138	
fnd*×*tbr					
IGC-01/17.n	20	1 (5)	IGC-01/17.5	87	5,44
IGC-02/200.n	2	0	--	--	--
IGC-02/201.n	8	0	--	--	--
Total	30	1 (3,33)		87	

*Owing to obtaining of berries and seeds by free pollination (self-pollinations) of clones precise calculate of seeds number and seeds number per pollination was not done

As a rule, interspecies F_1 hybrids, which were able to be cross with ***tbr*** dihaploids, were more robust as compared with wild parent (particularly for ***acl*** и ***fnd***) and they were of a transitional type from allotetraploids to diploid ***tbr*** in some morphological traits. The most of the F_1 ***acl***-based and some ***sto, plt, fnd*** –based hybrids had late tuber formation (November–December); as well as long-lasting dormancy of tubers (tubers of some ***sto***- and ***plt-*** based hybrids began to sprout only in late July–early August). Many of hybrids based on ***acl*** and some on ***sto*** and ***fnd*** did not form tubers at all.

The estimation of functional pollen fertility of some F_1 diploid hybrids showed that part of them were sufficiently fertile (pollen germination in vitro was more than 5%) for using them as mail parents in reciprocal cross combinations. Efficiency of hybridization by pollination of five ***tbr*** dihaploids with a mixture of pollen from hybrids based on ***acl*** and ***sto,*** was about the same as by pollination of interspecies F_1 hybrids with ***tbr*** dihaploids. It is varied from 0.6 to 35.6 seed per pollination (the mean value was 16.25).

Viability of seeds and plantlets and plantlet tuber formation of the first interspecies backcross generation increased in comparison with F_1, especially for hybrids based on ***acl*** and ***fnd,*** but remained lower than for intraspecies diploid ***tbr***-based hybrids.

It was obvious, that the formation of diploid interspecies hybrids is concerned with a loss of some part of the parental wild species genome. The analysis of introgression species-specific DNA-loci into genome of ***acl-***, ***sto-*** and ***plt***-based diploid hybrids was performed using. 13 highly polymorphic for potato RAPD-primers [7] and 12 SSR-primers [8]. Many species-specific loci from wild species were detected in diploid hybrids. (Table. 3). Considerable part of genetic material of wild species was inherited by offspring of backcrosses of these hybrids with ***tbr-***dihaploids.

Table 3. Presence and introgression level of wild species-specific loci in F_1 diploid hybrids between allotetraploid species and *S.tuberosum* dihaploids

Interspecies hybrids	Presence[1] of species-specific loci (%)	Introgression[2] level of species-specific loci (%)
acl ×***tbr***	4,6	22,57
sto ×***tbr***	5,43	35,76
plt×***tbr***	10,93	69,14

[1] Presence of species-specific loci: P= (number of species-specific loci / number of loci accounted)×100%.
[2] Itrogression level: (P of the hybrid / P of corresponding wild species) ×100%.

Use of SSR-markers made possible localization of some species-specific loci from wild species for the hybrids: IGC-02/199.2 (***acl***×***tbr*** F1) on chromosomes II , IV and VIII; IGC 01/19.10 (***sto***×***tbr*** F1) on chromosomes IV and VIII; IGC 01/19.12 (***sto***×***tbr*** F1) on chromosomes IV, VIII, XII; IGC 01/19.13 (***sto***×***tbr*** F1) on chromosomes IX, XII; IGC-04/6.n (***plt***×***tbr*** BC1) on chromosomes I, V, VII and IGC-04/7.n (***acl***×***tbr*** BC1) on chromosome VIII.

High level of introgression of species-specific loci, particularly of Mexican allotetraploid species of series *Longipedicillata*, shows the high breeding value of diploid interspecies hybrids and their offspring because of presence of genes from wild species. Such hybrids can be used for developing initial breeding potato material for disease and pest resistance. Genotypes with high resistance to PVX and PVY (ELISA estimation of field resistance was made in 2006 and resistance in deliberate infection of the material was performed in 2007) were selected during two-year field trials of BC1 and BC2 of ***acl-*** and ***sto***-based diploid hybrids. Some highly LB-resistant clones were selected among such hybrids during severe *Phytophthora infestans* epiphytotia of 2006 and in deliberate infection of the material in 2007.

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DEVELOPMENT OF THE DIPLOID BREEDING MATERIAL OF POTATO ORIGINATED FROM SOMATIC HYBRIDS BETWEEN *SOLANUM TUBEROSUM* L. DIHAPLOIDS AND WILD SPECIES FROM MEXICO *SOLANUM PINNATISECTUM* AND *SOLANUM BULBOCASTANUM*

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Diploid EBN1 potato species from Mexico *Solanum bulbocastanum* (***blb***) and *S.pinnatisectum* (***pnt***) are considered to be the most promising source of genes for breeding the initial material with high and durable LB-resistance. However, introgression of such genes into breeding material is complicated because of pre- and postzygotic incompatibility barriers between these species and *S. tuberosum* (***tbr***) [1 etc]. This problem can be solved in some extent by the use of somatic hybridization via protoplast fusion, which makes it possible to overcome any interspecies barriers. It has been used for production of the hybrids between ***tbr*** and various potato species, including ***blb*** and ***pnt***. Nonetheless, interspecies somatic hybrids in many cases are uncrossable with cultivated potato varieties [2 etc]. In particular, we failed to obtain viable seeds in crosses between tetraploid somatic hybrids ***tbr*** + ***blb***, ***tbr*** + ***pnt*** and highly fertile potato varieties or fertile ***tbr*** dihaploids producing 2n-pollen [3]. Meanwhile, production of somatic hybrids is only the first step in the introgression of the gene pool possessed by wild species into breeding material, as the primary hybrids require a subsequent breeding modification via backcrosses with ***tbr.***

The realization of approach to involve the valuable gene pool of Mexican potato species into the breeding are presented in this paper. The essence of this approach is the production of dihaploids ($2n= 2x= 24$) of the tetraploid somatic hybrids ***tbr+pnt*** and ***tbr+blb*** ($2n= 2x= 48$) and their subsequent backcrossing with ***tbr*** dihaploids [3].

The tetraploid somatic hybrids ***tbr+pnt*** (nos. 551, 552, 553, and 554) and ***tbr+blb*** (nos. 555, 556, and 557) produced by fusion of the protoplasts of dihaploids of ***tbr*** B-15 (***pnt*** hybrids), Gemma (***blb*** hybrids), and the corresponding wild potato species [4] were used as the initial material. The in vitro plants of somatic hybrids were kindly provided by Dr. L. Schilde-Rentschler (University of Tuebingen, Germany). Dihaploids were obtained by pollination of somatic hybrids by the pollen of the haploid producer *S. phureja* IvP35, which was marked with the mutation embryo spot. The haploid producer IvP35 was obtained from the International Potato Center (CIP, Peru). Anther culture and a combination of anther culture and haploid producer were used to produce dihaploids of ***tbr+blb*** hybrids. Highly fertile ***tbr*** dihaploids (2n= 2x = 24) were created and used for backcrossing dihaploids of somatic hybrids.

It was assumed that the homozygotes at *S* genes of ***tbr*** origin would be present among the dihaploids of somatic hybrids, which would be compatible as a female parent in crosses with c ***tbr*** dihaploids. This assumption was confirmed by our study of *tbr* + *pnt* (4*x*) hybrids. All the analyzed ***tbr*** + ***pnt*** hybrids produced berries with viable seeds being pollinated with the pollen of haploid producer *S. phureja* IvP35 (Table 1). However, we failed to obtain the dihaploids of ***tbr*** + ***blb*** (4*x*) somatic hybrids using this method. The only produced berry contained non-viable seeds.

Table 1. Results of hybridization between somatic hybrids (female) and the haploid producer S. phureja IvP35 (male)

Maternal form	Number of			
	pollinations	berries	seeds/berry	seeds/pollination
551 (***tbr+pnt)***	71	13	15,5	2,8
552 (***tbr+pnt)***	177	54	40,5	12,4
553 (***tbr+pnt)***	74	29	37,0	14,5
554 (***tbr+pnt)***	160	59	61,0	22,5
555 (***tbr+blb)***	25	1	25	1
557 (***tbr+blb)***	204	0	0	0

Ten seedlings with green-colored stems were chosen among the progenies obtained; five seedlings originated from the hybrid ***tbr*** + ***pnt*** 551 and five – from the hybrid ***tbr*** + ***pnt*** 554. Chromosome counting in these seedlings confirmed that they were diploids ($2n = 2x = 24$). Almost all obtained dihaploids developed well under greenhouse conditions, flowered lavishly and formed large amounts of pollen. They revealed the traits of both wild and cultivated parents and formed tubers; several clones had a high level of LB- resistance. All of the ten specimens hybridized readily with ***tbr*** dihaploids: berries with viable seeds were obtained from each crossing combination (Table 2). The pollen tubes of ***tbr*** reached the ovary without any signs of inhibition.

Table 2. Results of hybridization between dihaploids of somatic hybrids S. tuberosum + S. pinnatisectum (female) and dihaploids of S. tuberosum (male)

Dihaploids of somatic hybrids	*S.tuberosum* (2x)				Dihaploids of somatic hybrids	*S.tuberosum* (2x)			
	number of			pollen tube growth, score (points)		number of			pollen tube growth, score (points)
	pollinations	berries	seeds/ pollination			pollinations	berries	seeds/ pollination	
551-4	14	3	24,6	-	554-1	12	3	17,8	-
551-5	9	2	22,4	5	554-2	18	3	4,5	5
551-6	39	13	46,3	5	554-3	22	2	6,4	5
551-9	15	6	31	4	554-7	29	4	10,2	-
551-10	5	4	140,8	-	554-8	15	3	6,9	-

The semi-cultivated diploid species *S. phureja* evidently stands out among the remaining potato species from South America by its ability to overcome both the prezygotic and postzygotic reproductive interspecies barriers. In particular, very efficient expedient of rescue pollination in combination with *in vitro* culture of hybrid embryos is based on involvements of this species [5]. This ability was used for production of hybrids between potato varieties with different EBN. As it is evident from the results of our work, the prezygotic interspecific barriers between ***pnt*** and South American species are not so stringent as in the case of ***blb***. This underlies the success in obtaining seeds using pollination of ***tbr*** + ***pnt*** somatic hybrids by the pollen of haploid producer *S. phureja* IvP35 and the failure of obtaining seeds in the case of ***tbr*** +***blb*** hybrids. Apparently, the more universal yet more complex method of *in vitro* anther culture for decreasing the ploidy could be more appropriate in these cause.

We developed for this purpose the anther culture technique, which the most essential peculiarity is special conditions for growing anther donor plants, which increase androgenic ability of different potato genotypes [6]. This technique was effective in respect of ***tbr+blb*** somatic hybrids. All samples used in the experiment (555, 556 and 557) formed callus in anther culture. However, regenerated plants were obtained only for the clones 555 and 556 (in general, 26 plants). The Hybrid 555 was the best for androgenic ability in the experiment: 39% of explanted anthers produced callus able for plant regeneration.

Chromosome counting in 24 regenerated plants showed that most of them were mainly tetraploids. It was evident that they were doubled dihaploids of somatic hybrids. They had almost the same characteristics as the initial plants. However, inbred depression was visible: the plants were weaker, developed more slowly with delay in flowering and less flowering activity and they were more susceptible to virus and other infections. As the result, only 17 androgenic clones were suitable for hybridization with potato varieties and haploid producer IvP35.

Berries were obtained only in one combination of crosses between androgenic clones and potato cultivars (mixture of pollen from male fertile varieties was used). These berries contained shriveled seeds, which were not able to germinate in vitro. Estimation of pollen tube growth in pistil has shown that six androgenic clones did not have any inhibition of pollen tubes characteristic for initial somatic hybrids. Thus, production of doubled dihaploids of somatic hybrids made it possible to overcome prezygotic incompatibility between somatic hybrids ***tbr*** +***blb*** and cultivated potato. Obviously, these six androgenic clones were homozygous for S-genes of ***tbr*** origin.

The results of hybridization between androgenic clones of somatic hybrids and haploid producer IvP35 are presented in Table 3. The most androgenic clones set berries and seeds. Comparison of these data with pollen tube growth in crosses between androgenic clones and potato varieties indicated that clones, which did not set berries, had distinct pollen tube inhibition. This means that they apparently retain S-genes of ***blb*** origin. The berries contained not many plump seeds (от 3 до 32), however they had satisfactory germinating capacity being planted in soil (up to 50%) and *in vitro* culture (40-60%).

Table 3 Results of hybridization between androgenic clones of somatic hybrids S.tuberosum + S.bulbocastanum (4x) and haploid producer S.phureja IvP35

Androclones of ***tbr+blb***	Pollinator IvP35			Androclones of ***tbr+blb***	Pollinator IvP35		
	Number of				Number of		
	pollinations	berries	seeds/ pollination		pollinations	Berries	seeds/ pollination
555-1-1*	40	5	16	556-2-3	5	1	0
555-1-3	22	0	-	556-5-2	30	5	6
555-1-6	18	1	12	556-5-3	11	0	-
555-1-8	3	1	-	556-5-4	16	0	-
555-1-9	12	3	11	556-5-5	9	0	-
555-1-10	8	1	3	556-5-6	11	2	6
556-1-1	30	2	10	556-5-7	19	2	13
556-2-1	8	1	0	556-5-8	8	3	32

*Designation of androgenic clones: the first figure means the number of somatic hybrid, the second – the number of callus, the third – the number of plant regenerated from this callus.

The selection of putative dihaploids was carried out among 39 hybrids between androgenic clones 555-1-1, 555-1-9, 556-1-1, 556-5-2, 556-5-6 and IvP35 by means of chloroplast counting in leaf stoma guard cells. 7 plants were selected with 12,2 - 14,4 chloroplasts characteristic for diploid potatoes. Remaining plants were apparently triploids and tetraploids. Almost all of these plants were weak and did not form flowers. Only one dihaploid originated from androgenic clone 555-1-9 was rather vigorous and had good growth and flowering activity. As the result, we succeed in its hybridization with fertile ***tbr*** dihaploids. 15 flowers of this dihaploid were pollinated by the mixture of pollen from ***tbr*** dihaploids. 3 berries was formed and 23 plump seeds were planted in vitro. 17 seeds were germinated and formed 17 *in vitro* plants. All these plants were transferred to greenhouse where they formed the tubers. This dihaploid of somatic hybrid was homozygous for S-genes of ***tbr*** origin and its EBN was equal 2. The same results we obtained in respect of somatic hybrids ***tbr*** + ***pnt*** (see above). Thus, reduction of ploidy level of such 4x-somatic hybrids (EBN3) makes it possible to produce dihaploids with EBN2 which are crossable with ***tbr*** dihaploids.

PCR-analysis of the diploid breeding material obtained by above described method was carried out to estimate the introgression of genes of wild species. 13 highly polymorphic for potato RAPD-primers [7] and 12 SSR-primers [8] were used for this purpose. It was revealed that up to 20% of species-specific loci were retained in dihaploids of somatic hybrids (introgression level) (Table 4). Use of SSR-markers made it possible to localize some of ***pnt*** species-specific loci in 7 of 10 dihaploids of ***tbr*** + ***pnt*** on chromosomes: I (STM 1049), VI (STM0019) and XII (STM 0030). Species-specific locus of ***pnt*** on chromosome X (STM1106) was found in two such dihaploids.

Table 4. Presence and introgression level of species-specific loci in somatic hybrids S.tuberosum + S. pinnatisectum and S. tuberosum + S. bulbocastanum and their dihaploids

Hybrids	Presence[1] of species-specific loci (%).	Introgression[2] level of species-specific loci (%).
Somatic hybrids ***tbr*** + ***pnt*** (4x)	13,33	72,56
Dihaploids of Somatic hybrids ***tbr*** + ***pnt*** (2x)	4,28	23,35
Somatic hybrids ***tbr*** + ***blb*** (4x)	19,15	75,00
Androgenic clones of somatic hybrids ***tbr*** + ***blb*** (4x)	18,62	72,93
Dihaploid of somatic hybrid ***tbr*** + ***blb*** (2x)	11,70	17,02

[1] Presence of species-specific loci: P= (number of species-specific loci/number of loci accounted)×100%.
[2] Itrogression level: (P of the hybrid/P of corresponding wild species) ×100%.

Thus, new approaches on overcoming interspecies incompatibility between tetraploid somatic hybrids ***tbr+pnt, tbr+blb*** and cultivated potato were studied, which are based on decreasing of ploidy level of somatic hybrids by means of anther culture and use of haploid producer *S.phureja* IvP35. They make possible the production of diploid offspring of the somatic hybrids, which have the genetic material of wild parent and able to cross with ***tbr*** dihaploids.

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NEW APPROACH IN BREEDING POTATO BASED ON SELECTION OF PLANTS REGENERATED FROM UNREDUCED GAMETES IN ANTHER CULTURE

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The method of anther culture is usually using in plant breeding for decreasing the ploidy level of the initial material and producing doubled haploids. Anther culture of potato varieties can be used for transferring tetraploid material to the diploid level. However, tetraploid plants are regenerated in anther culture of potato varieties in many cases, which could be also interesting in breeding purposes [1 etc]. Gametoclonal variability of plants regenerated in anther culture well surpasses variability of plants regenerated in somatic cells culture (somaclonal variability), since it is based on gene recombinations during the gametogenesis rather then their mutations. Thus, some gametoclones can be used in breeding as the source of valuable characters. Disadvantage of such breeding for potato is that most tetraploid plants, regenerated in anther culture, are inferior to initial varieties in performance because of increasing the level of their homozygosity.

The decrease of productivity is main characteristic for tetraploid plants regenerated with chromosome doubling from normal (n=2*x*) pollen. Plants obtained in such cases are doubled dihaploids. At the same time, regeneration of tetraploid plants in anther culture can be from unreduced (2n=4*x*) pollen. It is known, that unreduced gametes have advantage over normal gametes in regeneration ability in diploid potato [2].

Different potato genotypes produce 2n-gametes with frequency conditioned to their meiotic mutations. The mutation *fs* (fused spindles) resulting in 2n-gamete formation of FDR-type (first division restitution) is of most interest for potato breeding. Formation of such gametes is not associated with essential decrease in their heterozygosity [3]. Therefore, tetraploid plants regenerated from anther culture of FDR unreduced gametes supposed to have the level of heterozygosity similar to the initial variety, inessential inbreeding depression and not to be inferior to initial variety in breeding value. At the same time, due to recombinogenesis, they can have the number of dominant alleles determining some breeding characters other than initial variety. Expression of correlated traits can be also different. As a result, such gametoclones can have valuable characters such as the improved tuber shape, earlier maturity, resistance to diseases and unfavorable factors of environment, improved combining ability and others [4]. Meiotic mutations resulting in formation of unreduced gametes of SDR-type (second division restitution) can be also of certain practical importance as decrease in heterozygosity for tetraploid forms is not so essential than for diploids.

Success in use of this method in practice can be possible on condition that: (1) the initial material has combination of high androgenic ability and satisfactory level of 2n pollen formation, (2) the use of effective technology of anther culture. Some effective methods of anther culture of potato varieties have been developed [5, 6]. However, formation of 2n-gametes in tetraploid potato varieties is not satisfactory studied. This problem was mainly investigated for diploids, as the tool for meiotic polyploidization of the initial material.

The aims of this study were investigation of 2n-gamete formation in wide range of tetraploid potato varieties, selection of varieties with different level of FDR and SDR 2n pollen production and estimation of plants regenerated from anther culture of such varieties.

The study of 31 potato varieties of Belarusian, Russian, German, Dutch and American breeding revealed great variability of microsporogenesis abnormalities resulting in 2n-pollen formation (Table 1). High frequency of dyad formation (more than 10%) had six varieties (19%), medium level (2-10%) had seven varieties (22%). In total, at about 40% of studied varieties were able to produce 2n-pollen. Thus, a broad spectrum of varieties can be used for improvement by anther culture from unreduced gametes.

Estimation of unreduced gamete formation performed in different years did not find significant fluctuation of these characters in most varieties. Only the variety Anosta had unstable expression of these abnormalities: 34% of dyads and 11% of triads in 2003, but both of these characters were less than 1% in 2005.

Table 1. Frequency of dyad and triad formation in microsporogenesis of tetraploid potato varieties

Variety	Nuber of analyzed cells	Frequency of formation (%)		
		tetrads	triads	dyads
511	1013	99,61	0,20	0,20
512	829	99,76	0,12	0,12
Adora	2284	93,43	0,96	5,60
Aksamit	1022	100	0	0
Alpinist	976	99,90	0	0,10
Anosta*	1991	81,67	4,42	13,91
Arnika	620	100	0	0
Aula	1089	89,72	2,11	8,17
Blakit*	2585	96,25	0,50	3,2
Bronnitski	1197	99,58	0	0,42
Charodej	613	99,84	0,16	0
Dubrava*	2673	57,35	1,35	41,30
Kolorit	625	94,56	1,12	4,32
Krynitsa*	1996	96,89	3,0	0,1
Lazurit*	2429	25,61	1,73	72,66
Lugovskoj*	4080	97,77	0,49	1,74
Nevskij	981	96,43	0,82	2,75
Nida	2254	92,15	0,49	7,36
Odissej	2214	99,50	0,05	0,45
Oreshenski	1220	95,82	3,69	0,49
Oressa	672	99,70	0,15	0,15
Pramen*	2859	99,69	0,01	0,2
Reserv	966	99,90	0	0,10
Russet Burbank	872	100	0	0
Sissi	504	100	0	0
Skazka	1278	99,22	0,63	0,16
Umatilla	981	34,96	21,92	43,12
Vesna Belaja	1374	98,84	1,09	0,07
Zagadka Pitera	2386	7,84	6,33	85,83
Zarevo	1212	100	0	0
Zhavoronok	907	99,23	0,77	0

* Generalized data of two years

Cytological study detected that the most varieties produced unreduced gametes of FDR type due to the mutations *fs* (fused spindles) resulting in dyad formation and *tps* (tripolar spindles) resulting in triad formation. It is known, that FDR pollen formation is characteristic for many wild species of potato and *S. tuberosum* dihaploids [7]. The results of our study show that many tetraploid potato varieties also produce FDR pollen with high frequency.

The mutation *pc (premature cytokinesis)* resulting in monad formation (unreduced gametes of SDR type) were detected in the variety Dubrava. Both *fs* and *pc* mutations were detected in variety Lazurit. Some varieties produced less than 1% of dyads and triads that was assumed to be incidental microsporogenesis disturbances, which might not associated with above mentioned mutations

The results of previous investigations indicated that even a low level of unreduced gamete formation can be sufficient for producing prospective genotypes by anther culture. For an example, the variety Lasunak produces about 4% of dyads and triads, nevertheless, at about half (from 44 to 62% regarding the character analyzed) of the population of plants regenerated in its anther culture had the same or even higher level of character performance as the original variety during three year tests [1]. Apparently, the higher frequency of FDR or SDR pollen formation in selected varieties will make the proposed approach more efficient.

The prerequisite for successful application of the proposed approach is sufficient androgenic ability of selected varieties. Callus formation and plant regeneration in anther culture of 11 varieties with different level of unreduced pollen formation we studied in 2007 (Table 2).

Table 2. Androgenic ability of potato varieties with different level of unreduced pollen formation

Variety	Level of dyad formation (%)	Number of anthers planted	Frequency of callus formation (%)	Number of plants regenerated	Frequency of regeneration (%)
Neptun	0,12	45	26,66	0	0
Veras	0,2	326	34,66	90	27,61
Promen	0,2	32	4,35	6	18,75
Blakit	3,2	128	31,82	9	7,03
Lugovskoj	6,34	128	47,66	20	15,63
Adora	5,60	70	48,57	0	0
Aula	8,17	28	60,71	0	0
Anosta	13,91	160	46,25	9	5,63
Dubrava	43,2	110	31,82	20	18,18
Lasurit	72,66	31	12,9	0	0
Zagadka Pitera	85,83	115	42,61	0	0

It was found that the varieties, which almost did not produce unreduced gametes, had better androgenetic ability in comparison with those, which produced unreduced gametes. Nevertheless, regenerated plants were obtained in anther culture of varieties producing 2n pollen, even in varieties with high level of dyad formation (Dubrava). The process of plant regeneration is continuing and we hope to obtain regenerated plants in remaining varieties.

PCR-analysis of plants regenerated in anther culture of the varieties Lasunak and Lugovskoj using highly polymorphic for potato 8 RAPD- and one ISSR-primers detected the similarity of regenerated plants with their initial varieties. Relatively low polymorphism of DNA profiles of regenerated plants confirmed their origin from unreduced gametes.

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ADVANCEMENTS IN THE APPLICATION OF MOLECULAR TECHNIQUES AT POTATO RESEARCH CENTRE, KESZTHELY, HUNGARY

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Introduction

Nowadays the most important aims of potato breeding are to develop new cultivars having complex resistance against abiotic and biotic stresses combined with higher quality and yielding potential.

Breeding for such complex characters by traditional techniques requires a large scale and very time consuming selection system. However the developments in molecular genetics could provide new opportunities (DNA-based markers, linkage maps) to speed up the breeding process. Linkage maps covering total genome of plants could be used to assign linked markers to different genes regulating agronomically important traits. The utilisation of linkage maps could also lead to the precise localisation of genes, and the very tightly linked markers could serve as starting points for cloning of the target genes itself.

Up till now, several publications reported the construction of the linkage maps of potato using different mapping populations and marker techniques (Bonierbale et al. 1988, Gebhardt et al. 1989, 1991, Jacobs et al. 1995; Meyer et al., 1998; Milbourne et al. 1998, Chen et al. 2001, Bradshaw et al. 2007). For now, more than 25 single dominant genes have been localized on these maps, most of them being pest-resistance genes, together with some quantitative trait loci (QTL) controlling yield and tuber quality traits (Barone 2004).

In nowadays breeding practices the molecular markers as selectivity tools are getting more frequently used providing higher accuracy for the selection programs (MAS) while saving time as well. At Keszthely a strict resistance breeding program for potato has been going on for more then four decades. The program released several varieties having good table quality combined with uniquely complex resistance traits to several important pathogens. The aim of our current work is to support this conventional breeding program by the application of modern molecular genetics namely linkage maps and markers based selection. In this paper we summarise the results we could earn during the last five years on this field.

1. project: Constructing of a linkage map in tetraploid potato

A mapping population consisting of 88 randomly chosen F_1 plants from a total of 506 available lines deriving from the simple cross between Hungarian cultivar White Lady (WL) and potato breeding line S440 has been developed. WL, the cytoplasmic male sterile partner possesses extreme resistance to viruses PVY, PVA and PVX, whereas it shows high field resistant against PLRV, it is resistant to nematode *G. rostochiensis* (*Ro1*, *Ro4*), and *P. infestans*. Moreover the segregating population could be suitable for the genetic analysis of different QTL's affecting some other important traits like processing quality.

To construct the linkage map the RAPD (Williams et al. 1990; Wels and McClelland 1990) and Intron-targeted (Choi et al. 2004) marker systems were applied, while to identify the different linkage groups (LG) two marker types - SSR (Milbourne et al. 1998) and functional markers (Chen et al. 2001) - were used.

RAPD analysis:

A total of 600 RAPD marker loci were detected in the F_1 progenies using 400 primer combinations. As many as 110 marker loci proved to be polymorphic between the two parents. Of these 48 were WL-specific and 62 S440 specific. From RAPD markers identified in female parent, 44 were scorable, from which 34 markers were classified as simplex and 10 as duplex. For male parent

S440, 58 RAPD markers were scored. Of these 53 segregated in a 1:1 ratio (simplex), and 5 segregated 5:1 ratio (duplex). Segregation patterns were observed for 4 RAPD loci between F_1 progenies but not between parents, suggested that they are present in double simplex form.

Intron-targeted strategy:

129 primer pairs were designed based on sequence information of different potato EST's, which – according to the results of homology search – will anneal in the exon enclosing the supposed intron region to amplify it. From 83 primer combinations which have been used so far, all of them amplified simple or multiple bands. Of these 19 bands showed length polymorphisms between the two parents. 11 primer pairs have been amplified only one fragment, but none of them showed polymorphism. These amplification products will be digested by different restriction endonucleases to identify sequence polymorphisms.

Identification of Linkage Groups:

So far, six functional marker (*Dbe*, *SutI*, *Tall*, *ShkB*, *Sus4*, *Ppe*) (Chen et al. 2001) have been tested between the two parents, and the mapping population, however only one (*SutI*) showed polymorphism after restriction digestion.

The amplification and polymorphism among the two parents were tested by 8 SSR primer pairs. Three of the primer pairs that amplified polymorphic products in the parents were subsequently tested on a segregation population. These markers mapped in three distinct LG's.

The built of partial linkage map:

Two linkage maps (maternal, paternal) were constructed using TetraploidMap (Hackett and Luo 2003) and JoinMap (Stam and Van Ooijen 1995) software. The tetraploid maternal genetic map has a total length of 273 cM comprising 19 linkage groups (8-2 markers/LG) and 9 isolated markers, while the paternal map consists 19 LG's (19-2 markers/LG) and 11 isolated markers with total map length 384 cM.

Since a high saturated linkage map could be suitable to identify markers linked to the target genes or QTL's, further work is currently under way to align a much higher saturated map, and to identify the LG's, which are necessary to localise the genes or QTL's determining important traits.

2. project: Marker-assisted selection for the gene *Ry*$_{sto}$

In our research institute - beside basic research programmes - the applicability of marker-assisted selection is examined too. Hence, here we report the application of PCR based markers in breeding process aiming to produce new potato cultivars having extreme resistance to PVY.

Three families (WL x S438, WL x Impala, Rioja x S440) were produced to combine good chips-quality with extreme resistance to PVY. In all of the crosses, the female parent carries the PVY resistance gene Ry_{sto} originating from *Solanum stoloniferum*. 189 F_1 hybrids deriving from three crosses were screened for two diagnostic PCR markers. The reliability of the marker based selection was tested by DAS-ELISA of the genotypes after artificial virus infection. The two markers used as diagnostic tool in this research were the *SCARysto4* developed by Cernák et al. (2008) and *STM0003-111* (Song et al. 2005). These markers are located on the two opposite side of Ry_{sto} gene, and have been mapped previously to 12 cM from each other (Cernák et al. 2008).

In a total 87 lines were identified in which both markers were present, and proved to be resistant by DAS-ELISA test. The numbers of lines in which both markers were absent and were susceptible to PVY in the serological tests were 73.

Recombination events were identified in 29 lines. Of these 18 lines contained only one of the markers irrespectively of the resistance type, while in 8 lines neither of the two markers was detectable, although they were determined as resistant by DAS-ELISA. Three recombinant lines were identified which proved to be sensitive to PVY, and contained both markers. These lines reveal the genotyping error that is – in this case – between 1.5-2 %.

Our results indicate that, the combined use of the *SCARysto4* and *STM0003-111* markers is very effective for the selection of potato genotypes carrying the Ry_{sto} gene. The reliability of the marker-assisted selection depends on the distance between gene and marker, or – like in this case – between marker and marker (Solomon-Blackburn and Barker 2001). The 12 cM distance detected between the two markers means a relative large distance, however our results indicate, that in spite of this the selection can be highly accurate due to the markers are locating on both side of the gene.

The *STM0003-111* and *SCARysto4* markers can be easily detected, and identified by agarose gel-electrophoresis, which makes their application fast and convenient. It can be conclude that marker-assisted selection for Ry_{sto} gene can be an efficient way of the identification of PVY resistant and susceptible individuals by the application of these two markers.

Since we observed the limited applicability of markers which have been developed in different genetic background, we feel it is necessary to develop new markers linked to different genes which are present in genetic background used in our institute. Further work is currently under way to develop the marker-assisted selection system to increase the efficiency the breeding process running in Potato Research Centre.

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MARKER ASSISTED SELECTION FOR INDIVIDUALS CARRYING *RPI-PHU1* GENE ENCODING LATE BLIGHT RESISTANCE IN DIPLOID AND TETRAPLOID POTATO

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Since the Great Irish Famine caused by potato late blight, *Phytophthora infestans* (Mont.) de Bary, the causal agent of this still important disease, become a prominent object of phytopathologists' studies. Breeding for potato cultivars resistant to late blight became one of the first goals of potato breeding efforts based on scientific fundaments [Świeżyński and Zimnoch-Guzowska, 2001].

In the beginning of the 20th century, eleven major resistance (*R*) genes against *P. infestans* were discovered in wild species *Solanum demissum* and named *R1-R11*. The breeders began to introduce those genes into cultivated potato gene pool [Stewart et al., 2001; Niederhauser et al., 1954]. As we now know *R* genes encode proteins from the NBS/LRR family and their numerous versions are found in all plants' genomes, where they are responsible for so called vertical resistance. The advantages of application of *R* gene-mediated resistance are the relative ease of introducing *R* genes into a breeding line and a usually high level of the obtained resistance. The biggest disadvantage is the limited durability of this kind of resistance. Recently a number of potato *R* genes for resistance to late blight have been cloned and the interest in applying them in practice is growing.

The search for novel sources of *P. infestans* resistance among wild and primitively cultivated *Solanum* species also takes place in Plant Breeding and Acclimatization Institute, Młochów. Among others, a major resistance gene originating most likely from *S. phureja*, *Rpi-phu1* was identified and mapped to chromosome IX of potato genome [Śliwka et al. 2006]. This gene was introduced by crossing to the *S. tuberosum* gene pool on the diploid level and then transferred to the tetraploid level by 2*x*-2*x* crossing. *Rpi-phu1* confers high level of resistance to *P. infestans* both in leaflets and in tubers, which is not correlated with late maturity. In order to offer the breeders the lines carrying *Rpi-phu1* together with a tool for distinguishing their resistant progeny we verified the possibility of using marker GP94 for the purpose of Marker Assisted Selection (MAS).

Materials and methods

GP94 is a simple, easy to detect PCR marker located about 6.4 cM from the *Rpi-phu1* gene in mapping population 97-30 [Śliwka et al. 2006]. In order to confirm its usefulness on the broad genetic background outside from the mapping population, we applied it as it is described by Śliwka et al. 2006 to following materials:

- two diploid potato populations (04-1, 04-2), in which the *Rpi-phu1* was segregating
- a tetraploid potato population (04-3), in which the *Rpi-phu1* also was segregating
- a group of 43 potato cultivars (Albina, Anielka, Balbina, Beata, Bekas, Bintje, Biogold, Bryza, Bzura, Cedron, Drop, Dunajec, Felka, Fregata, Fresco, Gandawa, Glada, Grot, Hinga, Hophely, Irga, Irys, Jagoda, Korona, Kuba, Lady Claire, Meduza, Nimfy, Omulew, Pasat, Perkoz, Romula, Sante, Sarpo Mira, Saturna, Skawina, Sokół, Ślęza, Triada, Umiak, Vistula, Vitara, White Lady), which potentially could be used as parental clones and therefore should not possess the marker allele $GP94_{250}$ linked to *Rpi-phu1* in order to enable MAS within their progeny
- 20 advanced tetraploid potato breeding lines segregating for *Rpi-phu1* presence
- 126 diverse diploid potato breeding lines segregating for *Rpi-phu1* presence
- 19 diploid potato hybrids containing *S. ruiz-ceballosii* (syn. *S. sparsipilum*) and *S. kurtzianum* germplasm, where the *Rpi-phu1* gene was absent.

The listed tetraploid and diploid breeding materials were tested phenotypically by a detached leaflet assay for resistance to *P. infestans*. Unselected populations 04-1, 04-2 and 04-3 were tested for the tuber resistance by the tuber slice test. Both in leaflet and in tuber slice test a 1-9 scale was used for scoring, where 9 means the most resistant [Śliwka et al. 2006].

Results

The results are shown in Table 1. In total 587 potato genotypes were tested with marker GP94, for all of them PCR products were amplified successfully (Fig. 1) and for 522 (88.9%) of them the marker test corresponded to the phenotype. In three unselected populations, the *Rpi-phu1* gene segregated in 1: 1 ratio. The individuals with *Rpi-phu1* gene were highly resistant (scores ≥ 8) in both tubers and leaflets (correlation coefficients ranged from 0.877 to 0.938 per population). The number of recombinants observed in those populations varied from 5.4 to 9.4%, which is not significantly different from 6.4 cM distance
observed in mapping population 97-30.

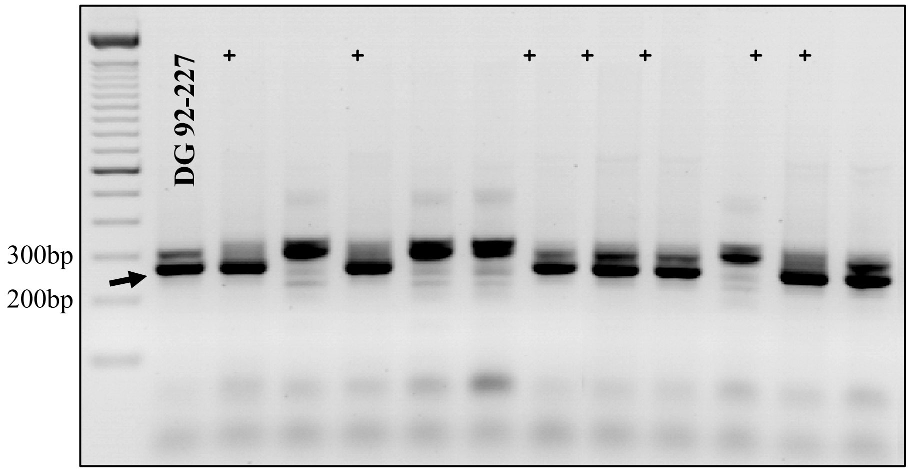

Figure 1. MAS for individuals carrying Rpi-phu1 with use of marker GP94 in tetraploid unselected population 04-3. DG -92-227 – positive control, "+" – resistant individuals, arrow indicates marker allele $GP94_{250}$ linked to the Rpi-phu1 gene.

Table 1. Screening of breeding materials with marker GP94.

Group of material	Ploidy	N	Resistance scored ≥ 8,0	Resistance scored < 8,0	Chi square 1: 1	r*l* correlation – leaflets/slices	Test GP94 N	N recom-binants	cM GP94 *Rpi-phu1*
Unselected pop.04-1[a]	2*x*	197	98	99	0.005 p < 0.943	0.938 p=0.000	129	7	5.4
Unselected pop.04-2 [a]	2*x*	192	92	100	0.333 p < 0.564	0.923 p=0.000	123	11	8.9
Unselected pop.04-3 [a]	4*x*	191	84	107	2.770 p < 0.096	0.877 p=0.000	127	12	9.4
Cultivars	4*x*	43	na	na	na	na	43	3	na
Breeding lines [a]	4*x*	20	9	11	na	na	20	0	na
Breeding lines [a]	2*x*	126	31	95	na	na	126	13	na
Hybrids (*ktz, rzc*)	2*x*	19	4	15	na	na	19	17	na

na- not applicable
[a] – resistance conferred by *Rpi-phu1*

Similar results were observed in groups of diploid and tetraploid breeding lines.

Within the group of 43 potato cultivars, only three (Beata, Hinga and Vistula) possessed the marker allele $GP94_{250}$ usually linked to the *Rpi-phu1*. That indicates that this allele is relatively rare in the cultivated potato gene pool and therefore can be useful for MAS.

However, in the group of diploid potato hybrids containing *S. ruiz-ceballosii* (syn. *S. sparsipilum*) and *S. kurtzianum* germplasm the marker allele $GP94_{250}$ was detected for 17 individuals out of 19, which all were not expected to contain neither the *Rpi-phu1* gene nor the marker allele linked to it. That indicates that the marker allele $GP94_{250}$ may be common in at least some of the wild species.

Summary

A simple PCR marker GP94 was amplified from diverse genotypes of both tetraploid and diploid potato. The marker allele $GP94_{250}$ was relatively rare in *S. tuberosum* gene pool and it was linked to the *Rpi-phu1* gene in diverse group of breeding materials. It can be a useful tool for tracking the *Rpi-phu1* gene in MAS.

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A BIOCHEMICAL AND MOLECULAR APPROACH TO STUDY THE EFFECTS OF DROUGHT ON DIETARY ANTIOXIDANTS IN NATIVE ANDEAN POTATO CULTIVARS (*SOLANUM TUBEROSUM* L.)

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Dietary antioxidants received increasing interest due to their prospective effects on the prevention of various diseases such as cancers and cardiovascular diseases. Significant levels of hydrophilic antioxidants, *i.e.* polyphenols and vitamin C, and moderate occurrence of lipophilic carotenoids and vitamin E have been reported in the staple crop potato. In this respect, the very diverse native Andean potato landraces are of particular interest from a nutritional point of view. Indeed, previous studies performed by our group stressed their high genotypic diversity as well as their high levels in health-promoting antioxidants such as zeaxanthin, alpha-tocopherol, chlorogenic acid and petanin as well as interestingly elevated levels in beta-carotene (Andre *et al.*, 2007a and 2007b).

The potato crop is known to be very sensitive to water stress. Moreover, in the current context of global warming, increases in temperature will be unavoidably linked to increase of drought. If the negative impact of water stress on tuber yield is quite known, little information is available concerning the nutritional quality of potato tubers under drought conditions. In the present study, the effects of drought stress on dietary antioxidants were investigated using a selection of five native Andean potato cultivars under field conditions. On the one hand, the main carotenoid, vitamin E, vitamin C and phenolic compounds were surveyed by means of high performance liquid chromatography. Hydrophilic antioxidant capacity was also analysed using the Oxygen Radical Absorbance Capacity (ORAC) assay. On the other hand, the expression of the genes involved in the biosynthesis of antioxidants was studied using real-time RT-PCR. We focused particularly on the genes involved in the carotenogenesis and in the phenylpropanoid pathway (biosynthesis of the polyphenol compounds).

Whereas the vitamin C contents were not affected by the drought treatment, significant modifications of the levels of carotenoids, vitamin E and polyphenols were observed in cultivar-dependent manner. The candidate gene approach to study the expression level of the antioxidant-related genes also revealed both cultivar-dependent and water-status dependent variations.

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BREEDING FOR DROUGHT AND SALT TOLERANCE IN POTATO

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Drought and salt stress are responsible for considerable crop loss worldwide. Therefore crops that are tolerant to drought and salt stress are called for. In addition, the increasing need for arable lands and the competition of bioenergy crops as well as the drainage of natural resources by modern agricultural practices demand for food crops that are agronomically viable on suboptimal and marginal grounds.

Potato is one of the major food crops but cultivated potato is relatively sensitive to drought and salinity stress.

At Wageningen UR Plant Breeding we are elucidating the genetic basis for drought and stress tolerance traits in potato using the mapping populations SHxRH and CxE. SHxRH serves as a reference population for which an extensive genetic map is available, with a matching physical map that runs to completion and the genome sequence pending (Potato Genome Sequencing Consortium). CxE is a mapping population that has been extensively characterized for morphological and quality traits.

Our strategy involves extensive phenotyping of the CxE population for salinity and drought tolerance, as well as extension of the CxE map with molecular markers targeted at candidate genes for drought and salt tolerance. These molecular markers include markers for single candidate genes (LEA5, Dehydrins, DREB genes) as well as candidate gene families. For the gene families, we have used the Motif-directed profiling approach to generate markers in Myb genes, WRKY genes and peroxidases, and a profiling approach for AP2 transcription factors is under development. In addition, we are currently analyzing a 384 SNP illumina Goldengate array with both CxE and SHxRH. The CxE map now consists of a collection of 40 well-distributed SSRs, 100-200 AFLP markers and more than 200 markers in gene families. The majority of these markers are also mapped in SHxRH, allowing us to make optimal use of the extensive genetic and genome sequence data of this reference population.
The CxE population will be characterized for morphological traits (including root properties) and physiological traits and biochemical parameters related to abiotic stress tolerance (ΔC discrimination, chlorophyll fluorescence, Na^+ and K^+ content) in a controled greenhouse environment. The physiological/biochemical/phenotypic data will be used with the marker data to identify QTLs for drought and salt tolerance. Interesting QTLs will be followed up by map-based cloning approaches using the genomics data available for the SHxRH population.

The CxE population is also grown in multiple environments around the world, including trials in South America Finland, and China. This enables assessment of Genotype x Environment with the QTLs found in CxE.

THE POTATO PHYSICAL AND SEQUENCE MAP AND ITS APPLICATION FOR MAPPING AGRONOMICALLY IMPORTANT TRAITS

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A fingerprint physical map is available from BAC clones of the diploid potato genotype RH89-039-16. This physical map is being used as the framework for BAC-by-BAC sequencing of the complete potato genome by the Potato Genome Sequencing Consortium (PGSC). Fourteen hundred BAC contigs have been anchored to the twelve chromosomes of potato by AFLP markers from the ultra-dense genetic map (Van Os et al., 2006). The BAC tiling paths that extend from these seed contigs are being sequenced by the participating countries of the PGSC on a chromosome-by-chromosome basis. By linking QTL data to these genetically mapped potato sequences, it will be possible to identify candidate genes for genetic variation in agronomically important traits, and to develop markers for breeding.

The Netherlands are sequencing chromosomes 1 and 5. These sequences will be used to analyse traits that have been mapped to these chromosomes. Of primary interest is the earliness QTL region on chromosome 5, where currently an improved physical map is being constructed on the basis of the newly obtained sequence data.

Reference:

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SEQUENCE ANALYSIS OF A NEW CZECH POTATO LEAFROLL VIRUS (PLRV) IZOLATE

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Abstract

Sequence analysis of the new Czech PLRV isolate was performed using PLRV specific primers covering the whole virus genome. The primers were derived from the sequences of known PLRV izolate (GenBank accession number AF453388) and the reverse trascription (RT) and PCR amplification were done using these PLRV specific primers. The preliminary comparison with other known PLRV izolates showed high level of nucleotide sequence similarity and the same genomic organization. The positions of ORFs (partial P0, P1 – P8) and partial 3`-UTR was identified. The nucleotide sequence reported will apear in the GenBank database under the accession number EU313202.

Introduction

Potato leafroll virus (PLRV) is the type member of the genus *Polerovirus* which belongs to the family *Luteoviridae*. This virus is especially dangerous in warmer areas of potato cultivation, where its natural aphid vector *Myzus persicae* (Sulzer) occurs in higher amounts and early in the growing season. Yield reduction in potatoes by PLRV may reach 80-90% in susceptible cultivars. The quality of tubers of some cultivars can also be reduced because of the net necrosis (Gilbert, 1928). This virus is mainly restricted to phloem tissues, causing the stem and apical leaves to roll. Its host range is mainly restricted to solanaceous plants [*Physalis floridana* (Rydb.), *Datura stramonium* L., *D. tatula*, *Lycopersicon esculentum* (Mill.), *Nicotiana clevelandii* and *Nicandra physaloides*] including potato *Solanum tuberosum* L., but also some plants of other families can be infected by PLRV (*Amaranthus caudatus*, *Capsella bursa-pastoris*, *Celosia argentea*, *Gomphrena globosa*, *Montia perfoliata*, *Nolana lanceolatus* and *Sisymbrium altissimum*) (Thomas, 1993; Ellis, 1992; Fox et al., 1993). Infected seed-potato is the main source of PLRV dissemination and in the field the virus is transmitted in a persistent non-propagative manner by more than ten different aphid species, *Myzus persicae* Sulz. being the most efficient and important vector (Harrison, 1984).

The RNA genomic sequences of several PLRV isolates have been determined (e.g. Mayo et al., 1989; Van der Wilk et al., 1989; Keese et al., 1990). The detailed sequence analysis has been done afterwards on three isolates from Brazil (De Souza-Dias et al., 1999), nine isolates from Tunisia (Khouadja et al., 2005) and seven further isolates (Guyader and Giblot-Ducray, 2002). Resembling characterization of Czech isolates is still missing. The RNA genome of PLRV consists of a ~ 5.9 kb positive-sense single-stranded RNA that is on the 5´-terminus covalently coupled to the small (7kDa) VPg protein and encapsidated in 25 to 30nm diameter isometric particles. There is no poly A sequence on the RNA 3´-terminus. The sequence of PLRV revealed eight main open reading frames (ORFs) numbered from 0 to 7. The ORFs are organized into two gene blocks separated by a small intergenic region (197nt). First three ORFs (ORF 0-2) on 5´-terminus are translated directly from genomic RNA, whereas the 3´-located genes are expressed from 2 subgenomic RNAs (sgRNA1 – 2.7 kb and sgRNA2 – 0.8 kb). The 5´-proximal ORF0 encodes a P0 protein (28 kDa) essential for PLRV genome replication (Sadowy et al., 2001), ORF1 codes for a proteinase-containing polyprotein P1 (70 kDa), whose self-cleavage releases VPg (Prüfer et al., 1999) and ORF1/2 encodes motifs typical for RNA-dependent RNA polymerases (RdRp, 118 kDa) translated by rarely occuring ribosomal frameshift from ORF1 (Mayo and Ziegler-Graff, 1996). Within the P1 coding region but in a different reading frame was identified a 5 kDa replication-associated protein 1 (Rap1) important for viral multiplication (Jaag et al., 2003). SgRNA1 encodes the 23 kDa coat protein P3 (Mayo et al., 1989), the 79 kDa readthrough protein expressed by occasional translational readthrough of a CP amber termination codon (Bahner et al., 1990; Brault et al., 1995) and the 17 kDa protein responsible for virus movement between cells (Tacke et al., 1993). SgRNA2 encodes proteins of 7.1 kDa and 14 kDa, the roles of which remain unknown, although the 14 kDa protein has nucleic acid binding properties

(Ashoub et al., 1998). This paper describes our preliminary results dealing with the sequence analysis of one new Czech PLRV izolate.

Material and Methods

Virus Source and immunocapture RT-PCR (IC RT-PCR):

The isolates of PLRV were collected from seed-potato cultivars evaluated in post-harvest certification trials. Infected potato plants were detected by ELISA and transplanted into aseptic *in vitro* conditions and there were further maintained in original host potato plants.

Immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) was used to generate cDNA fragments of PLRV RNA. The tubes were coated with 50µl anti PLRV IgG (Primediagnostics) diluted 1:1000 in coating buffer for 3h at 37°C. The wells were then washed (3 x 100µl PBS+T) and 50µl of the homogenate from PLRV infected potato leaves in extraction buffer (1:10) was added. The samples were incubated overnight at 4°C and washed again three times with PBS+T buffer. To obtain overlapping cDNA fragments the reverse transcription and PCR amplification with M-MLV reverse transcriptase (Promega) and Taq polymerase (Fermentas) following manufacturer's recommendations were done, using PLRV specific primers. PLRV izolat, accession number AF453388 (GenBank), was used as a template for deriving of different primer sequences.

Cloning and Sequencing:

The sequences were obtained by both sequencing of PCR products as well as fragments cloned into pUC57T/A (Fermentas). Sequencing was performed using an ALFexpressII sequencer with the autoRead sequencing kit (AP Life Science). Sequence analysis was done using DNASTAR (Lasergene) and multiple alignments on the internet using CLUSTAL W (Thompson et al., 1994). Contig from overlapping sequenced cDNA fragments was generated. The obtained sequence was deposited in the GenBank.

Results and Discussion

The nucleotide sequence of 5771nt covering the most of genomic RNA of the new Czech PLRV izolate was obtained and the preliminary comparison with other known PLRV izolates was performed. The sequence had a high level of similarity (93-99%) and the genomic organization of the Czech PLRV izolate was the same as in the case of other known PLRV izolates. Based on the multiple sequence alignments the positions of ORFs (partial P0, P1 – P8) and partial 3`-UTR were identified. The obtained sequence was deposited in the GenBank under the accession number EU313202. This PLRV isolate is maintained in *in vitro* virus collection with code name VIRUBRA 1/047 (http://www.vurv.cz/), Databases, Plant Medicine, Viruses, Collection of potato viruses and homologous antibodies.

Conclusion

This contribution summarizes our results concerning the sequence analysis of one new Czech PLRV izolate. We found out the high level of sequence similarity between Czech izolate and other known PLRV izolates and identified the positions of ORFs and 3`-UTR in the Czech PLRV izolate genome. There is a growing need for alternative method of virus desease control by achieving of higher resistance to PLRV in in potato cultivars. Therefore, this analysis may contribute to exploit alternative method identifying the regions suitable for construction of transgenic potato plants resistent to PLRV.

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DNA METHYLATION PATTERNS ASSOCIATED TO *IN VITRO* SHOOT REGENERATION OF *SOLANUM* SPECIES

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Somaclonal variation is known to be caused by both genetic and epigenetic mechanisms (Osborn *et al.*, 2003). The former are based on changes in DNA sequence, the latter alter gene expression without a DNA sequence change. DNA methylation is one of the most frequent epigenetic events associated to somaclonal variation. In this work, we aimed to gain insight into the effects of *in vitro* tissue culture and of chromosome doubling on DNA methylation in regenerants of Solanums using MSAP (Methylation-Sensitive-Amplified Polymorphism) (Xiong *et al.,* 1999) technique. This method relies on the different sensitivity to cytosine methylation of a pair of isoschizomers (*Hpa*II and *Msp*I). We studied two diploid (2n=2x=24) clones of *Solanum commersonii* (cmm) and *S. tuberosum* (tbr), and their *in vitro*-regenerated genotypes. In particular, MSAP analysis was performed on 8 *in vitro* regenerated tetraploids (4 from cmm and 4 from tbr), 4 regenerated diploids (4 from cmm and 4 from tbr), and on the parental genotypes they derived from. Six primer-enzyme combinations allowed the detection of polymorphisms of interest within the genotypes for each species (figure 1).

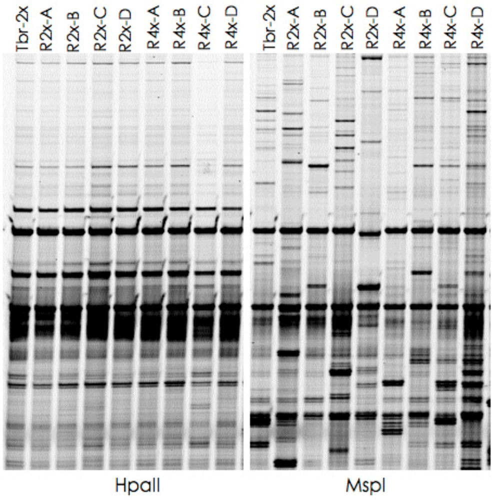

Figure 1: Example of MSAP fingerprinting performed on 2x S. tuberosum (tbr-2x) and its 2x (R2x) and 4x (R4x) regenerants using the enzyme combinations EcoRI-HpaII (left) and EcoRI-MspI (right). The E_{TCCA}/ M_{ACG} primer combination was used.

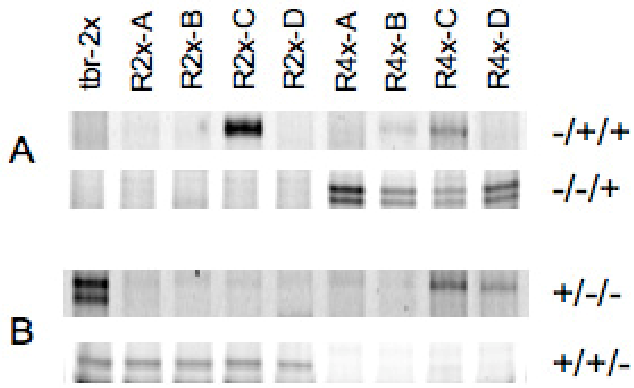

Figure 2: Example of different A) gain-type (-/-/+; -/+/+) and B) loss-type (+/-/-; +/+/-) banding patterns observed on S. tuberosum genotype (tbr-2x) and its 2x (R2x) and 4x (R4x) regenerants.

We compared the MSAP profiles of 2x *S. tuberosum* (tbr-2x) and *S. commersonii* (cmm-2x) with their 2x (R2x) and 4x (R4x) regenerants. For each genotype, polymorphic fragments were recorded as present (1) or absent (0). MSAP markers were reckoned and classified into two main categories of changes: “LOSS”, disappearance of parental MSAP fragments; “GAIN”, appearance of novel MSAP fragments. In particular, we differentiated gains occurring both in 2x and 4x regenerants (class – + +) and losses occurring both in 2x and 4x regenerants (class + – –) (figure 2). As for *S. tuberosum*, we observed more loss-type, than gain-type (–/+/+) patterns (table 1). Similarly, in *S. commersonii* regenerants the loss-type patterns were more frequent than the gain-type ones (table 2). In our study, as indication of the polyploidization-induced genome variation, we also considered those MSAP markers occurring in all 4x but not in 2x regenerants (gain-type pattern –/–/+) as well as those absent only in all 4x but not in 2x regenerants (gain-type pattern +/+/–) (table 1). We called them “diagnostic bands”, in that the constant disappearance/appearance of such fragments at 4x level may suggest that they can be associated with polyploidization. As for *S. tuberosum*, we observed only 1 diagnostic band for the "–/–/+" class and no one for the "+/+/–" class. As for *S. commersonii*, 4 patterns showed gain of fragments in 4x regenerants (class –/–/+) and 3 patterns showed the loss-type (–/–/+). These results suggest that wild *S. commersonii* is more prone to change its genome after polyploidization than *S. tuberosum*. Further analysis with larger sample size are necessarily to confirm this hypothesis.

Table 1. Results from MSAP analysis of 4x and 2x regenerants from S. tuberosum and S. commersonii. The number of MSAP markers, "gain-type" (–/+/+;–/–/+) and "loss-type" (+/–/–; +/+/–) banding patterns are reported.

Species	Total bands no.	GAIN no. (%)		LOSS no. (%)	
		–/+/+	–/–/+	+/–/–	+/+/–
S. tuberosum	518	26 (18.5)	1 (0.2)	18 (3.5)	0
S. commersonii	842	176 (21.0)	4 (0.5)	201 (23.9)	3 (0.3)

Our results showed that chromosome doubling *per se* do not change considerably the methylation status of the genome. In fact, few polymorphic fragments detected were associated to polyploidization. As expected, we found that *in vitro* tissue culture lead to great changes in the *Solanum* epigenome. This finding is deemed very important since cultivated potato and its wild relatives often undergo to *in vitro* manipulation and this may alter agronomic as well as qualitative traits.

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COMMUNITY RESOURCES FOR HIGH THROUGHPUT GENOME MAPPING AND DIVERSITY ANALYSES IN 1EBN POTATO SPECIES

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Efficient access to genetic variability is important for breeding programs. Wild potato species represent rich genetic resources for potato improvement. The USDA potato Genebank maintains more than 5,000 accessions of 135 potato species. Included are 20 closely related diploid potato species classified collectively as "superseries *Stellata*" that are of particular interest as sources of resistance to biotic and abiotic stress (Table 1). Among potato species, crossability is predicted by the endosperm balance number (EBN), with sexually compatible species sharing a common EBN. Most of the *Stellata* species are 1EBN, making them sexually incompatible with cultivated potato (4EBN). But genes from these species can be accessed using bridge crosses, somatic hybridization, and gene cloning and transformation. To improve access to agriculturally significant genes from *Stellata* species we have initiated an effort of comparative structural genomics using the Diversity Array Technology (DArT) marker platform (Jaccoud et al. 2001). DArT is a high throughput, microarray based

Table 1. Superseries Stellata

Series[a]	Species	EBN	Reported Disease Resistance[b]
I. Morelliformia	*S. clarum*	?	Early blight, PVM, Verticillium
II. Bulbocastana	*S. bulbocastanum*	1	Blackleg, Bacterial wilt, Early blight, Late blight, PLRV, PVM Ring rot, Verticillium, Wart
III. Pinnatisecta	*S. cardiophyllum*	1	Late blight, Ring rot, Verticillium, Wart
	S. pinnatisectum	1	Black leg, Early blight, Late blight, PLRV, Ring rot
	S. jamesii	1	Blackleg, PLRV, PVY, Ring rot, Verticillium
	S. tarnii	1	Late blight, Ring rot
	S. trifidum	1	Late blight, PLRV, Ring rot, Verticillium
IV. Polyadenia	*S. polyadenium*	1	Blackleg, Late blight, PLRV, PVA, PVM, Ring rot, Verticillium
V. Commersoniana	*S. commersonii*	1	Bacterial wilt, Blackleg, Early blight, Ring rot, Verticillium
VI. Circaeifolia	*S. circaeifolium*	1	Bacterial wilt, PVM, PVS, Rhizoctonia, Wart
VII. Lignicaulia	*S. lignicaule*	1	Fusarium wilt, Rhizoctonia, Verticillium, Wart
VIII. Olmosiana	*S. olmosense*	?	
IX. Yungasensa	*S. chacoense*	2	PVA, PVF, PVX, PLRV, Verticillium

[a]Classification scheme for superseries *Stellata* of Hawkes (1990) with modifications after Rodriguez and Spooner (1997), Lara-Cabrera and Spooner (2004), and Spooner et al. (2004).

[b]Resistance data from personal experience, published accounts (Austin et al. 1993; Helgeson et al. 1998; Micheletto et al. 2000; Naess et al. 2000; Song et al. 2003; Vossen et al. 2005; Vossen et al. 2003), and USDA Potato Genebank website.

technology capable of detecting thousands of polymorphic markers dispersed throughout the genome. Because data generation entails nucleic acid hybridization to a common set of immobilized markers, DArT data generated in one study are directly comparable to data from a second. DArT markers are thus well suited for cross-population, cross-species, and cross-laboratory applications.

We have developed a DArT array suitable for mapping and diversity analysis in 1EBN potato species. This array was developed in two phases. First, as part of an international potato consortium, a preliminary array comprised of approximately 7,500 markers was constructed from diverse potato germplasm including five *Stellata* species: *Solanum commersonii, S. bulbocastanum, S. polyadenium, S. chacoense*, and *S. pinnatisectum.* Application of this array to examine species relationships within *Stellata* (Fig. 1) demonstrated concordance between DArT marker data and previous molecular and morphological studies. This preliminary study confirmed the utility of DArT markers for analysis of wild potato germplasm and assisted in selecting polymorphic 1EBN genotypes as parents of mapping populations. In the second phase of this project, we are now expanding the potato DArT array. The current version of the array consists of approximately 16,500 markers, with approximately 9,500 markers derived from *Stellata* species. The array has been especially enriched for markers originating from 1EBN species including the late blight resistant *S. bulbocastanum* and the cold tolerant *S. commersonii*.

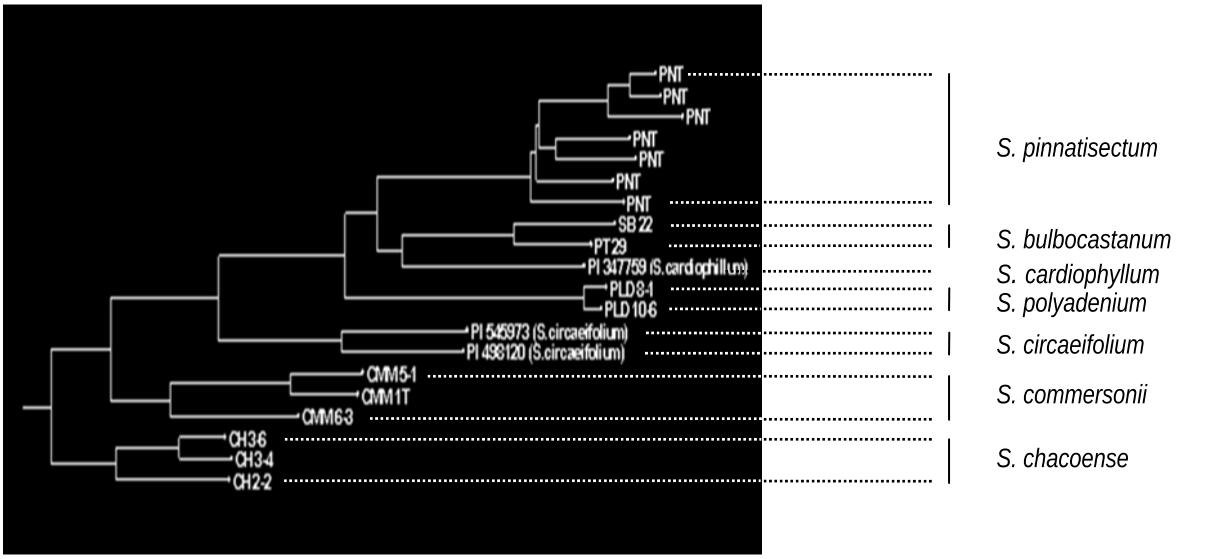

Fig. 1. Phylogenetic analyses of wild potato species using DArT markers.

Evaluation of 20 *Stellata* genotypes using a ~7,500 marker DArT array. In this preliminary analysis, genotypes cluster according to species. Species affinities predicted by DArT data are concordant with predominant classification schemes based on molecular and morphological data (Table 1).

Next, we will use DArT markers for detailed phylogenetic analysis of *Stellata* species and for comparative mapping amongst these species. Currently, marker analysis of two mapping populations, one derived from *S. bulbocastanum* and a second from *S. commersonii*, is ongoing. Results to date predict that at least 1,000 DArT markers will be mapped in *S. commersonii* and at least 750 DArT markers will be mapped in *S. bulbocastanum.* An additional interspecific mapping population is also being constructed. The resulting three maps, constructed from a common set of DArT markers, will facilitate comparison of genome structure within the 1EBN potatoes. By sequencing mapped DArT markers, we will be able to extend comparisons of genome structure to the cultivated potato and tomato, both current subjections of genome sequencing, via *in silico* analyses. Finally, array hybridization-based anchoring of DArT markers to BAC libraries will allow the incorporation of BAC clones containing candidate disease resistance genes into DArT-based linkage maps. This will facilitate resistance gene mapping, marker aided selection, and gene cloning. In support of this effort, large-scale phenotypic analyses are also ongoing. Phenotypic tests include evaluation of all available accessions of *S. commersonnii* (61 accessions) and *S. chacoense* (133 accessions) for foliar resistance to *Phytophthora infestans* (late blight). Preliminary data reveal that while many of the accessions are susceptible, some useful levels of resistance exist.

Once construction and testing of the potato DArT array is complete, the array and all marker sequence data generated will be available to the international research community. Please contact the authors for details.

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INVESTIGATING THE ROLE OF ANTIBIOTIC PRODUCTION IN *ERWINIA CAROTOVORA SSP. CAROTOVORA*

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Abstract

One of the most important potato pathogens are species of *Erwinia carotovora,* which cause soft rot of tubers and blackleg of stems. Some *Erwinia* isolates produce the antibiotic carbapenem (1-carbapen-2-em-3-carboxylic acid), thought to have role in bacterial niche protection in elimination of competitive bacteria from the site of the infection. The impact of the antibiotics produced on potato associated bacterial communities during *Erwinia* infections is poorly understood. Therefore, we aimed to compare the extent to which potato rhizosphere associated bacteria can take advantage and multiply *in planta*, utilising the nutrients released by an *Erwinia* infection, when the infecting *Erwinia* is either an antibiotic producer or is a carbapenem knock-out strain (Ecc-carC$^-$). Our results indicate that rhizosphere inhabiting bacteria can indeed take advantage of an *Erwinia* infection and multiply in planta even in the presence of carbapenem. In addition, we have shown that the ability to synthesise the antibiotic is essential for ensuring the persistence of the *Erwinia* in the fully rotted tuber.

Introduction

Erwinia carotovora is a Gram negative bacterial phytopathogen that is the causative agent of plant soft-rots and the potato disease blackleg (stem rot). Two subspecies are recognised *Erwinia carotovora* ssp. *carotovora* (Ecc) and *Erwinia carotovora* ssp. *atroseptica* (Eca), *Eca* has a narrower host range, primarily being a pathogen of potato (Perombelon MCM, 2002). Some Ecc strains produce a broad spectrum β-lactam antibiotic, 1-carbapen-2-em-3-acid carboxylic acid (carbapenem), thought to have a role in niche defence by eliminating competing bacteria from sites of infection (Whitehead *et a*l, 2002). Production of carbapenems by Ecc is controlled in a cell density dependent manner, requiring the accumulation of the small diffusible molecule N-(3-oxohexanoyl)-L-homoserine lactone (OHHL) in the growth medium (Baiton *et* al, 1992,) and *in planta* during infections. In Ecc*,* the synthesised 3-oxo-C6 HSL binds to an activator protein CarR, initiating the transcription of the carbapenem biosynthetic and resistance gene cluster (*car A-H*) (McGowan *et* al, 1995, McGowan *et* al, 1997, Welch *et* al, 2000). Studies regarding carbapenem antibiotic production by *Erwinia* species were carried out mainly in culture media (reviewed by Coulthurst, S.J., 2005) and therefore little is known about these mechanisms under natural circumstances such as during plant infection. The impact of the antibiotics produced by *Erwinias* during infection on potato associated bacterial communities (rhizosphere bacterial communities and endophytic bacteria) as well as the role of antibiotics produced it has been only anticipated (Whithead *et* al, 2002). Thus, we aimed to analyze the extent to which potato rhizosphere associated and endophytic bacteria can take advantage of an *Erwinia (*Ecc*)* infection and multiply *in planta* utilizing the nutrients released when the infecting *Erwinia* is either an antibiotic producer or is a carbapenem knock-out strain.

To investigate the role of carbapenem produced in niche protection or niche selection, two Ecc strains were created; a GFP/luxABCDE labelled carbapenem producing (EccB6Tn5-GFP/luxABCDE) and a labelled carbapenem knock-out (EccB6car$^-$GFP/luxABCDE) strain. These labelled strains allowed us to monitor the number and the proportion of erwinias at the beginning and throughout a four day infection. The results indicate that during the initial stages of the infection antibiotic production has little or no effect on either the total number of bacteria or the proportion of erwinias, however, as the infection progressed, numbers of the non-carbapenem producing erwinias declined far more rapidly than the carbapenem producing strain. These finding may indicate that, in *Erwinia* species, carbapenem antibiotic production contributes to the ecological fitness of the producing strain.

Results

Introduction of the gfp/*luxABCDE* marker genes in wild type *Erwinia carotovora* ssp. *carotovora*

In this study gpf/*luxABCDE* marker genes were introduced by Tn5-*gfp/luxABCDE* insertion mutagenesis into the parent Ecc strain's genome in order to create a carbapenem producing labelled strain (EccB6-Tn5-GFP/luxABCDE). A carbapenem negative strain (EccB6-carC-GFP/luxABCDE) was created from the same parent strain by homologous recombination of the marker genes within the carbapenem biosynthetic and resistance gene cluster (*carA-H*, McGowan *et* al, 1997), maintaining functional carbapenem resistance genes. Disruption of carbapenem genes was confirmed by demonstrating the tagged strain's inability to inhibit growth of a carbapenem super-sensitive *E. coli* tester strain (ESS, Baiton *et* al, 1992). Similar T-streaks with the wild type and tagged strain indicated that the expression of the downstream genes had not been compromised.

Carbapenem production promotes *Erwinia* survival in presence of the competitors

Labelling the *Erwinia* strains with *gfp/luxABCDE* marker genes allowed the growth and relative number of the two *Erwinia* strains to be monitored throughout the 4 day infection. To do so, we used a tuber slice system in which washed, non sterile tubers were sliced and inoculated with one of the labelled *Erwinia* strains and culturable bacteria were counted following tuber maceration and plating. In order to monitor the population dynamics during the course of *Erwinia* infection, enumeration of bacteria at different time points (starting at time point 0, immediately after inoculation) of the infections was carried out. Colony forming unit (CFU) counts of culturable bacteria recovered from the infected tuber slices was determined per whole tuber slice. To establish the proportion of GFP/*lux*ABCDE labelled erwinias from the total number of culturable bacteria, plates were double counted. The total number of cultivable bacteria was first established, and then the luminescent signals of the erwinias were acquired. Immediately after inoculation (time point 0 hours), the total number of bacteria/tuber slice was between 2-6x10^7 CFU for both treatments and 1.4x10^7 for the control non-inoculated tuber slices. The proportion of inoculated erwinias from the total number of cultivable bacteria was between 5-10% for both infections. No significant differences between the treatments or the control tuber were recorded at this time point (ANOVA, P>0.05). Twenty four hours after the inoculation the total number of bacteria increased ca. 20 fold in both *Erwinia* infections. The proportion of erwinias 24 hours after inoculation was 85% for the EccB6carC-GFP/lux and 88% for the EccB6Tn5-GFP/lux infection. No significant differences were observed between the number (ANOVA, P=0.4952) or the proportions (ANOVA, P=0.5926) of the two Ecc strains, as well as between the total number of cultivable bacteria (ANOVA, P>0.05). After 48 hours, the numbers of the two erwinias were 9.85x 10^8 for the carbapenem knock-out strain and 2.17x10^9 for the carbepenem producing strain. This does not represent a significant difference (ANOVA, P=0.3961) between the two infections. However, significant differences (ANOVA, P= 0.0043) between their proportion of the total cultivable bacterial population were recorded. At this time point, the proportion of the non-carbapenem producer erwinias decreased from 85% to 3% whilst in the case of the carbapenem producer strain it decreased from 88% to 18%. Seventy two hours after infection, both the number (1.5x10^8) and proportion (0.63%) of the non-carbapenem producer continued to decrease; 96h after infection, the light producing carbapenem negative strain could no longer be detected. In the case of the carbapenem producer strain, numbers stayed relatively constant (4.72x10^9 at 72h and 4.92x10^9 at 96h) in the later stages of the infection. As a proportion of total bacteria, the carbapenem producing strain showed a slight (non significant) increase, due to a slight reduction in non *Erwinia* species, rather than due to a second round of *Erwinia* growth. In both infections the total number of bacteria increased throughout infection, but no significant differences were observed for this between the two treatments at any of the time points (Figure 1).

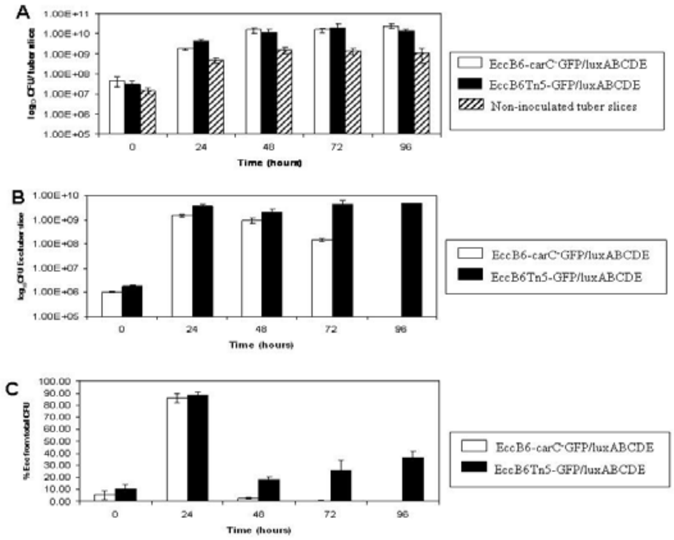

Figure 1. (A) Total number of culturable bacteria (CFU) isolated from tuber slices infected with either EccB6-carC-GFP/luxABCDE or EccB6Tn5-GFP/luxABCDE, and non-inoculated tuber slices at 0, 24, 48, 72 and 96 hours after inoculation. (B) Total number of culturable Erwinias (EccB6-carC- GFP/luxABCDE and EccB6Tn5-GFP/luxABCDE) isolated from tuber slices at 0, 24, 48, 72 and 96 hours after inoculation. (C)Proportion of the Erwinias from the total CFU of cultivated bacteria over the four day infection.

Discussions

The results described here indicate that, in *Erwinia* species, carbapenem antibiotic production contributes to the ecological fitness of the producing strain. It has been suggested (Whitehead *et* al, 2002) that carbapenem production serves to prevent competing bacteria utilizing the nutrients released following the infection-derived maceration of the plant tissues. However, the inability to produce carbapenem did not significantly affect the competitiveness of the erwinias over the first 24 hours; equal amounts of rotting were seen in both sets of tuber slices and both *Erwinia* strains reached similar cell numbers. It is in the later stages of infection that a pronounced difference emerges, the total number of carbapenem producing *Erwinia* remains steady whilst the non producer declines. This suggests that the ability to synthesise antibiotic assist for long term survival in the presence of other bacterial species.

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TRANSGENIC RESEARCH

INTROGRESSION IN *SOLANUM* BY SOMATIC HYBRIDIZATION

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Somatic hybridization is one of more successful technique for introgression into cultivated potato the desirable traits from incompatible wild species. Samples of potato somatic hybrids with agronomically important traits derived from wild species were selected in combinations with tuberous species *S. circaeifolium* (Mattheij *et al.,* 1992; Oberwalder *et al.,* 1998), *S. phureja, S. commersonii, S. bulbocastanum, S. pinnatisectum, S. verrucosum* (rev. Orczyk *et al.,* 2003), *S. tarnii* (Thieme *et al.,* 2004, 2008), and nontuberous ones *S. brevidens* (rev. Orczyk *et al.,* 2003), *S. etuberosum* (rev. Orczyk *et al.,* 2003; Thieme *et al.,* 2004), *S. nigrum* (Zimnoch-Guzowska *et al.,* 2003). Crucial moment in somatic hybridization is the problem of fertility of obtained somatic hybrids and their capacity to generate the viable progeny in crosses with cultivated potato. Generation of sexual progeny of somatic hybrids is of great original importance irrespective of the presence of agricultural useful traits because it enhances the gene pool of the classical breeder.

In our experiments somatic hybridization by protocol of chemical fusion was used in different combinations including diploid or tetraploid cultivated potato and wild species or interspecific sexual hybrids. Sample of tetraploid *S. tuberosum* 78563-76 set berries with viable seeds by free pollination in the field and greenhouse. Accessions of dihaploid cultivated potato cv. Lasunak (LDH) and 86-6 (dihaplid cv. Lasunak × *S. chacoense*) had intensive flowering but formation of berries depended on the year. The lines of collection *Solanum in vitro* from biotechnology laboratory of our Centre were used as wild partners. As rule they were obtained by incorporation of the true seeds into culture *in vitro* with the following selection on resistance to domestic isolates of potato pathogens. Used lines of Mexican species *S. bulbocastanum* (Sb), *S. cardiophyllum* (L8), *S. polyadenium* (L39-2) and Bolivian *S. circaeifolium* (E50-2) possessed the resistance to haulm late blight in the test with cassettes independently of year of evaluation. The plants from *in vitro* culture were grown in 64 cell cassettes with soil and in 4-5 weeks after planting were sprayed with *Phytophthora infestans* suspension. Selected lines of *S. polyadenium* formed berries with viable seeds but *S. bulbocastanum* only bloomed without berry set. The lines of nontuberous wild partners *S. etuberosum* (E55-1, E56-1) *S. caripense* (L30), *S. etuberosum* × *S. brevidens* (L49-2), *S. brevidens* × *S. etuberosum* (L48-3) as rule were selected on resistance to PVY and PLVR by grafting and on resistance to black leg in the laboratory test on bouquets of green stems of greenhouse-grown plants. All samples of nontuberous parents excepting L49-2 of sexual hybrid *S. etuberosum* × *S. brevidens* bloomed but never formed berries. L49-2 did not form flowers during eight years of growing in the greenhouse.

The regeneration of plants and identification of hybrids was observed in thirteen combinations of fusion. The selection of hybrids was conducted mainly by isoenzymes of peroxidase and in some case by isoenzymes of malatdehydrogenase, esterase, profiles of soluble proteins or RAPD patterns.

Morphological characters more than 200 somatic hybrids were described in the greenhouse or in the field during 3-5 vegetative seasons. Morphological characters of parents were displayed in plants of interspecific somatic hybrids of all combinations in different degree by traits of leaf (shape), rarely of corolla and stem and presence of anthocyanin coloration in different parts of plant. The formation of tubers in all somatic hybrids with tuberous wild species excepting *S. circaeifolium* was like as in a cultivated parent. The flowering of different intensity was varied from year to year and observed in all combinations, somatic hybrids with *S. circaeifolium* excluding. The formation of berries from free pollination or (and) in backcrosses was absent in combinations with *S. jamessii* and sexual nontuberous hybrid *S. brevidens* × *S. etuberosum*. Tnd true seeds were extracted from berries of seven combinations:

SB - *S. tuberosum*, 4x (78563-76) + *S. bulbocastanum*

DL - *S. tuberosum*, 2x (LDH) + *S. bulbocastanum*
F - *S. tuberosum*, 4x (78563-76) + *S. polyadenium*
P – *S. tuberosum*, 2x (LDH) + [*S. polyadenium* + (*S. etuberosum* × *S. brevidens*)]
C - *S. tuberosum*, 4x (78563-76) + *S. caripense*
2D – 86-6, 2x + *S. etuberosum*
4D – 86-6, 2x + (*S. etuberosum* × *S. brevidens*)

However production of true seeds for somatic hybrids of combination P was observed in a single case, when one seed was extracted from backcross berry. As rule their berries were seedless. Thus somatic hybrids of this combination didn't posses the fertility. Somatic hybrids DL set annually a great number of berries, but they rarely contained the seeds. In the previous years only two seedlings were regenerated from two backcrosses. But under the conditions of 2007 there was obtained one berry from free pollination which contained the seeds (92) like that as it was usually observed in classical breeding.

Fertile somatic hybrids have been selected for combinations SB, C, F, 2D and 4D. They were crossed with tetraploid *S. tuberosum* and fruits with viable seeds which germinated *in vitro* and *in vivo* were obtained. In all these combinations, 4D excepting, both parents bloomed and one of them or both (F) set berries. Successful backcrosses with SB hybrids were obtained when maternal plants were grown on a brick. This technique induces prolonged and intensive flowering and hinders formation of tubers. Numerous sexual progeny which includes first or (and) second backcrosses was received for F, SB, 2D and 4D somatic hybrids.

The somatic hybrids of combinations SB were resistant to haulm and tuber late blight during 9 years (1999-2007) when grown in the field without fungicides (Yakovleva *et. al.,* 2007). The late blight development in Belarus was epidemic in 2000, 2001, 2006 and moderate epidemic was in 2003-2005. According to PCR data obtained with specific primers, the genomes of somatic hybrids carry the genes *R1* from 78563-76 comprising the germplasm of *S. demissum* and *RB* from *S. bulbocastanum*. It seems that the stable late blight resistance of somatic hybrids depends on *RB*.
The stable resistance to haulm and tuber late blight was also observed in generative progeny of SB hybrids for three last years including epidemic 2006 year (Table 1).

Table 1. Field assessment of yield and resistance to late blight of generative progeny of somatic hybrids SB without defence by fungicides in 2006 – epidemic development of Phytophthora infestans. Resistance to haulm blight on 28.08.2006 and tuber blight (whole tuber in laboratory test) is recorded on a 1-9 scale (1 - susceptible, 9 – resistant). Replication is 20-40 bushes for genotype. SD – standard deviation.

Genotype	Parentage	Score for late blight		Tuber weight (g/bush ± SD)
		Haulm blight (mean ± SD)	Tuber blight (mean ± SD)	
Sp2-5	SB5-2 free pollination	9,0 ± 0	9,0 ± 0	1000 ± 60
Sp2-8		8,4 ± 0,1	9,0 ± 0	988 ± 74
Sp2-10		8,7 ± 0,1	9,0 ± 0	1030 ± 70
Sc5-4	SB5-2 × (88.34/14 and 88.16/20)	7,8 ± 0,1	6,6 ± 0	762 ± 53
Sc5-7		9,0 ± 0	9,0 ± 0	740 ± 60
Sc5-8		7,8 ± 0,2	9,0 ± 0	998 ± 73
Sc5-19		9,0 ± 0	9,0 ± 0	637 ± 62
Sc6-72	SB7 × (88.34/14 and 88.16/20)	9,0 ± 0	9,0 ± 0	1007 ± 106
Sc6-87		8,4 ± 0,1	9,0 ± 0	850 ± 124
Sc6-97		8,9 ± 0,1	9,0 ± 0	744 ± 68
Sc6-120		9,0 ± 0	9,0 ± 0	1066 ± 108
Suzoriye	late cv. - standard	3,7 ± 0,2	6,0±1,4	842 ± 48

The presence of *S. bulbocastanum* genetic material in sexual progeny of somatic hybrids SB was confirmed by 4-6 bands specific to genotype Sb on the profiles of soluble proteins.
Important character of somatic hybrids with nontuberous wild partners *S. etuberosum* (2D) and *S. etuberosum* × *S. brevidens* (4D) and their sexual progeny is the ability to set tubers. The number of tuberous genotypes in populations of somatic hybrids 2D and 4D is very small but the situation has

changed in the first sexual progeny (Table 2).

Table 2. The assessment of resistance to PVY, PLVR, black leg of stem and tuber and first field evaluation of yield for generative progeny of somatic hybrids 2D and 4D in the 200. Evaluation of resistance to viruses by grafting in greenhouse, black leg – in laboratory for stem from greenhouse and tubers from field. R – resistant, MR – moderate resistant, S – susceptible, nt – not tested.

Genotype	Parentage		Tuber weight (g/bush ± SD)	Resistance to disease			
	♀, somatic hybrid	♂		PVY	PLVR	Black leg	
						Stem	Tuber
86-6	Cultivated partner		356,8 ± 38,2	S	R	MR	MR
3-1	2D-8-5	962100-12	488,0 ± 75,8	R	nt	R	R
12-3	2D-177-3	Uladar	946,0 ± 84,6	R	R	R	R
16-1	2D-265-3	Uladar	1453,8 ± 160,4	R	R	R	R
16-3			1776,8 ± 234,0	R	R	R	R
17-1	2D-265-6	Uladar	1280,0 ± 119,4	R	R	R	R
51-30	2D-4-7	free pollination	331,7 ± 101,1	nt	nt	nt	nt
265-3-6	2D-265-3	free pollination	937,8 ± 57,2	S	R	MR	S
35-11	2D-265-3	free pollination of 265-3-6	660,0 ± 238,9	R	R	nt	S
265-8-1	2D-265-8	free pollination	937,8 ± 77,6	S	S	R	MR
26-3	4D-11-3	962100-12	711,2 ± 43,8	nt	nt	MR	MR
26-11			607,1 ± 92,6	R	nt	R	R
26-12			635,7 ± 73,9	nt	nt	R	R

The genotypes resistant to PVY, PLVR, black leg of stem and tuber, if the hybrid formed tubers, have been selected in the populations of somatic hybrids of combinations 2D and 4D. Resistance to PVY and PLVR was estimated in the test with grafting of analyzed genotype on the tomato plants contaminated by PVY or the potato plants contaminated by PLVR. Symptoms of diseases on the wilding and alive grafts were described in 28-35 days after grafting. At the same time the plants of graft and wilding were tested by ELISA. Virus and (or) stem and tuber black leg resistant genotypes were also selected in sexual progeny of somatic hybrids 2D and 4D.

The presence of nontuberous genetic material in sexual progeny of somatic hybrids 2D and 4D was confirmed by presence of 1-2 bands specific to genotype of nontuberous sample in peroxidase isoenzymes.

Thus it seems that: 1) ability to form tubers in interspecific somatic hybridization is like to that of cultivated parent; 2) ability to set tubers in combination with nontuberous wild parent may be restored in the first generative progeny of somatic hybrids; 3) for revealing fertility of somatic hybrids it is necessary that one or both parents set berries with viable seeds; 4) somatic hybrids with *S. bulbocastanum* had the stable resistance to haulm and tuber late blight that may be connected with presence of *RB*.

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STUDY OF AGRONOMICAL CHARACTERS OF SOME POTATO LINES GENETICALLY MODIFIED FOR RESISTANCE TO COLORADO BEETLE ATTACK

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In Romania, the potato is an important vegetable, an essential food element for humans and animals, consumed during the whole year. Annually, the potato is cultivated on an area covering about 280,000 hectares. The production per unit area is small, equivalent to only 30% of that in some western European countries.

Harvest yields are drastically affected by the attack of pathogen agents and pests. Among the last, the most harmful is Colorado beetle, which has become a quarantine organism in Western Europe, whilst in Romania has two or even three generations per year.

The cultivation of genetically engineered potato, otherwise meaning, the Bt potato, would make possible the protection of crops without the application of insecticides with beneficial effects on the environment and human health. The results of a study regarding the economic impact have shown, without a doubt, that the application of potato Bt technology in Romania would make possible savings of up to 10 million USD, of which 4 million would represent the cost for insecticides and 6 million the cost for treatments (Otiman et al., 2004)

Redsec and Coval varieties belonging to Târgul Secuiesc Research Station have been genetically modified at University of Agricultural Sciences Timisoara for the *cry3A* gene expressing resistance to Colorado beetle attack (Frantescu et al., 2003; Badea et al., 2004) Several transgenic lines derived from two varieties were evaluated for different agronomic and resistance traits, in the greenhouse conditions.

MATERIAL AND METHOD

The biological material studied was represented by 14 genetically modified lines of Romanian potato variety – Redsec and 14 genetically modified lines of native variety -Coval.

The researches regarding the biology and agronomical characteristics were performed in greenhouse conditions and using potted plants while investigations made with regard to attack level of Colorado beetle were made both in greenhouse and laboratory conditions by artificial infestation using larvae and adults.

Assessment of resistance traits of different lines against Colorado beetle was achieved by attributing notes depending on attack intensity and frequency. The results obtained from biometrical measurements relating to different morphological and yield characters were statistically processed obtaining main estimative values and subsequently the significance of differences between genetically modified lines and parental forms on the ground of variance analysis and t test was established (Ciulca, 2006).

RESULTS

Regarding plant height, it has been observed that lines of Redsec variety registered values between 27,67 cm and 33,60 cm with variation amplitude of approximately 6 cm and average variability while for Redsec variety, plants measured 31,27 cm.

Ramification degree for Redsec variety lines showed values between 2, 53 and 3,40 with an amplitude of 0,87 due to a very large variability within lines. As to number of leaves per plant, in case of Redsec variety lines, there were registered values between 12,80 and 22,80 with an amplitude of 10 and increased variability within potato lines.

Concerning plant height, it has been observed that lines of Coval variety presented values between 31,20 cm and 35,40 cm with reduced variation amplitude of only 3,8 cm and average variability while Coval variety registered plant heights of 33,24 cm. Ramification degree for the lines of Coval variety registered values comprised between 3 and 5 with amplitude of 2 due to very large variability within potato lines. In case of Coval variety lines, the leaf number per plant registered values comprised between 13,60 and 23,20, with amplitudes of 9.60 and increased variability within potato lines.

Table 1.The significance of differences between lines of Redsec variety concerning tuber weight per plant

Line (var.)	Tuber weight (% comparing the control)	Relative value (%)	Difference comparing the control	Significance
Redsec	100,00±25,61	100	0,00	Control
2R	118,69±13,17	118,69	18,69	-
3R	158,43±28,63	158,43	58,43	*
4R	84,172±8,75	84,17	-15,83	-
5R	117,33±15,23	117,33	17,33	-
6R	118,28±38,02	118,28	18,28	-
7R	179,08±30,60	179,09	79,08	**
8R	84,172±8,75	84,17	-15,83	-
9R	125,06±25,14	125,06	25,06	-
10R	126,39±25,95	126,39	26,39	-
11R	140,79±17,71	140,79	40,79	-
12R	96,982±10,22	96,98	-3,02	-
13R	95,128±15,28	95,13	-4,88	-
14R	152,03±13,04	152,04	52,03	*
15R	114,15±18,99	114,15	14,15	-

$LSD_{5\%}$	$LSD_{1\%}$	$LSD_{0.1\%}$
47,57	63,33	82,42

Referring to mean tuber yield per plant comparing with Redsec control variety, 71% of lines deriving from it registered superior values. Three of these, 3R, 7R and 14R attained superior yields comparing the control and were also statistically covered. Only two lines (4R, 8R) attained considerably inferior yields comparing the control, of 16% without statistical cover.

Table 2. The significance of differences between lines of Coval variety concerning tuber weight per plant

Line (var.)	Tuber weight (% comparing the control)	Relative value (%)	Difference comparing the control	Significance
Coval	100,00±4,79	100,00	0,00	Control
1C	113,20±26,27	113,20	13,20	-
2C	156,59±26,70	156,59	56,59	*
3C	82,70±22,07	82,70	-17,30	-
4C	112,92±16,05	112,92	12,92	-
5C	112,44±21,64	112,44	12,44	-
6C	101,10±3,68	101,10	1,10	-
7C	109,02±8,09	109,02	9,02	-
8C	107,67±16,70	107,67	7,67	-
9C	83,19±25,46	83,19	-16,81	-
10C	83,39±7,82	83,39	-16,61	-
11C	79,25±15,24	79,25	-20,75	-
12C	94,68±17,70	94,68	-5,32	-
13C	108,42±6,37	108,42	8,42	-
14C	55,67±8,49	55,67	-44,33	0

$LSD_{5\%}$	$LSD_{1\%}$	$LSD_{0.1\%}$
43,14	57,44	74,75

With respect to mean tuber yield per plant comparing with control –Coval variety, 57 % of lines rising from it registered superior values. Four of these, 1C, 2C, 4C and 5C have presented yield increases of 15% but statistically covered only for potato line 2C. The lowest tuber yield was registered by 14C line which proved to be with 45% significantly inferior comparing the control.

Virus attacks were registered for all studied potato lines, without conspicuous and significant differences concerning attack intensity between lines and original varieties.

Taking into consideration the attack intensity generated by Colorado beetle on lines of Redsec varieties irrespective of method used for artificial infection in greenhouse and laboratory conditions, it has been noticed several distinct significant differences between lines relating to resistance traits.

Comparing with Redsec variety, 11 of 14 potato lines proved to be significantly superior regarding the resistance to Colorado beetle attacks. In case of 2R, 3R and 11R lines, CRY IIIA gene that confers resistance to Colorado beetle was not expressed. The remaining lines rising from Redsec variety have registered attack intensity values between 9,00 % for 6R and 41,67 % for 4R. Approximately 65% of the lines rising from this potato variety have shown attack intensity lower than 20%, the most resistant lines relating to this trait proved to be: 6R, 9R, 7R.

Table 3. Significance of differences between lines of Redsec variety concerning Leptinotarsa decemlineata attack intensity

Line (var.)	Attack intensity (%)	Relative value	Difference comparing the control	Significance
Redsec	100,00±0,00	100,00	0,00	Control
2R	100,00±0,00	100,00	0,00	-
3R	100,00±0,00	100,00	0,00	-
4R	41,67±14,24	41,67	-58,33	000
5R	13,33±3,33	13,33	-86,67	000
6R	9,00±3,79	9,00	-91,00	000
7R	11,00±2,08	11,00	-89,00	000
8R	15,33±7,33	15,33	-84,67	000
9R	10,33±7,42	10,33	-89,67	000
10R	18,00±11,14	18,00	-82,00	000
11R	100,00±0,00	100,00	0,00	-
12R	21,00±9,71	21,00	-79,00	000
13R	12,67±6,23	12,67	-87,33	000
14R	12,33±2,66	12,33	-87,67	000
15R	13,33±4,41	13,33	-86,67	000

$LSD_{5\%}$	$LSD_{1\%}$	$LSD_{0.1\%}$
14,51	19,54	25,98

In case of lines rising from Coval variety, it has been also observed significant differences under the aspect of resistance level against Colorado beetle attack considering the heterogeneity of experimental conditions and also due to inter-individual variability between pest larvae and adults used to perform artificial infection.

Comparing to Coval variety, all potato lines rising from it have registered very significantly inferior attack intensity. The attack level on studied lines presented values between 5,33 and 13,33 %, approximately 65% of lines registering attack intensity below 10%. The most resistant lines proved to be: 6C, 12C, 4C, and 14C. The lines deriving from Coval variety have registered an attack intensity considerably reduced than lines resulting from Redsec variety.

Table 4 Significance of differences between lines of Coval variety concerning Leptinotarsa decemlineata attack intensity

Line (var.l)	Attack intensity (%)	Relative value	Difference comparing the control	Significance
Coval	100,00±0,00	100,00	0,00	Control
1C	7,67±1,45	7,67	-92,33	000
2C	7,67±1,45	7,67	-92,33	000
3C	13,33±13,33	13,33	-86,67	000
4C	6,67±0,88	6,67	-93,33	000
5C	11,33±6,84	11,33	-88,67	000
6C	5,33±2,33	5,33	-94,67	000
7C	9,00±1,00	9,00	-91,00	000
8C	9,33±0,67	9,33	-90,67	000
9C	16,67±7,26	16,67	-83,33	000
10C	11,67±1,67	11,67	-88,33	000
11C	13,33±3,33	13,33	-86,67	000
12C	5,67±0,67	5,67	-94,33	000
13C	9,33±0,67	9,33	-90,67	000
14C	6,67±2,02	6,67	-93,33	000

$LSD_{5\%}$	$LSD_{1\%}$	$LSD_{0.1\%}$
11,53	15,53	20,65

CONCLUSIONS

On the ground of obtained results there have been selected the following lines: 7R, 14, 11R, 9R, 10R; which showed a high significant attack intensity inferior to Redsec variety, and attained at the same time tuber weight per plant considerably superior to this variety.

Relating to Coval variety, there have been identified the following lines: 2C, 1C, 4C, 5C, 7C; which attained tuber weight per plant superior to original variety due to a high significant resistance superior to it.

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THE TRANSGENE CODING FOR THE COLD-INSENSITIVE PHOSPHOFRUCTO-KINASE AFFECTS THE REDUCING SUGAR CONTENT IN POTATO TUBERS

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Storage of potato tubers at low temperature brings many advantages including natural control of sprout growth, minimization of physiological weight loss, and reduction of losses caused by storage diseases. Main disadvantage represents phenomenon known as low-temperature sweetening. To decrease reducing sugar (RS) accumulation we transformed potato plants with a bacterial gene for cold-insensitive phosphofructokinase (*LbPFK*) (Branny *et al*, 1993). The gene is under control of the tuber specific promoter *pB33* directing the transcription of patatin gene family I (Rocha-Sosa *et. al.*, 1989). During tuber development the patatin gene family I represent 99 % of all patatin mRNAs in tuber cells (Blundy *et al.*, 1991). The gene *LbPFK* is actively transcribed in tubers but not in the leaves (Navrátil *et al.*, 2007).

Experiments with two transgenic potato varieties expressing unmodified *LbPFK* provided encouraging results. While the tubers from untransformed control plants steadily accumulated RS during the cold storage those from transformed plants regardless the variety were characterized by the gradual decrease in the RS content. After long period (160 d) of cold storage in one case the RS decreased by more than 60% compared to the control (Navrátil *et al.*, 2007).

For further investigation we prepared a new variant of the gene *LbPFK*. The sequence surrounding the initiation codon was changed to the consensus sequence of dicots and some rare codons at the 5´ end of the gene were replaced by frequently used plant codons. In addition to the originally used variety Kamýk two other Czech potato varieties (breeder Vesa Velhartice, Plc.) were transformed to verify the effect of the modified *LbPFK*. Testing of all transformed plants in the field trials is in progress. It is important to know the RS values for the field grown plants. As was demonstrated on biochemical phenotyping of potato tubers, the values of different biochemical markers for transgenic and wild type soil-grown tubers formed independent clusters, while the values of the same plants grown in vitro formed clusters very close each to other (Roessner *et al.* 2002).

The field trial also includes the limited number of transgenic lines bearing the unmodified *LbPFK*. Some of them are evaluated in field trials since 1998. The RS content in their tubers was previously measured using the Luff-Schoorl's method (Navrátil *et al.*, 2007) determining all reducing substances represented in potato tubers mainly reducing sugars. At present, we can compare separately values of sucrose, glucose, and fructose after their separation by HPLC. The storage conditions for harvested tubers remain unchanged. Tubers are stored up to five months at +4° C and RS content is determined either immediately after removal of tubers from the store or after their reconditioning at +16°C for 18 days, as established in food industry practice. In our previous study the decrease in the RS due to reconditioning was remarkable for all the years and all the periods of the cold-storage testing. It is also evident that the decrease diminishes with the storage prolongation.

The RS values of the transgenic cultivar Kamýk exceed in some years the values recommended for the chipping cultivars (‹ 0.33 % FW; Duplessis *et al.*, 1996). We expect that for the cultivar Vladan the RS content could be steadily lower than this recommended value because of the low natural sugar content. The RS values for transgenic tubers (just before cold-storage) range from 0.23 % to 0.1 % FW.

Some authors argue for the role of the cold lability of enzymes catalyzing the conversion of fructose 6-phosphate to fructose 1,6-biphosphate in cold-induced sweetening (e.g. Malone *et al.*, 2006). We have verified the contribution of the cold-insensitive phosphofructokinase which can supply for the negligible activities of the cold-sensitive potato phosphofructokinases (Dixon *et al.*, 1981; Hammond *et al.*, 1990). Moreover, the pH optimum of the cytosolic form of the plant enzyme is around 6.5 and the enzyme activity is sensitive to the low pH values, which are achieved in potato tubers already after one week of the cold-storage (Bredemeijer *et al.*, 1991). On contrary, the glycolytic enzymes of *Lactobacillus* are insensitive to the low pH values (Le Bras *et al.*, 1998). It seems that the activity of the cold-insensitive phosphofructokinase can restrict the RS values in cold-stored tubers.

Our further investigation will focus on the role of a cold-insensitive pyruvate kinase.

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UNRAVELLING STARCH GRANULE MORPHOLOGY IN POTATO

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Starch is the major form in which carbohydrates are stored and is present in almost all plant organs at one or another time during development. Storage starch is formed in amyloplasts as dense granules ranging in size from 1 uM to over 100 uM. They are composed of an essential linear glucose polymer and a branched glucose polymer termed amylose and amylopectin respectively.

Knowledge about starch biosynthesis is required to be able to modify starches for industrial or food uses. This knowledge has been acquired by studies in a broad range of plants using both mutants and reverse genetic approaches. In this way the function and impact of a number of the essential genes in the starch biosynthetic pathway has been assessed.

Modification of starch biosynthesis pathways holds an enormous potential for tailoring granules or polymers with new functionalities. Until recently a lot of effort was put into investigating the individual components in the starch biosynthetic pathway. Both enzymes involved in synthesizing and degrading carbohydrate polymers have been down-regulated using different inhibition approaches. In most cases this were single gene knock out attempts although in some cases two or even three genes were addressed at once. Examples of this are the production of amylose-free starch, high amylopectin starch and starch with an increased degree of branching. This has lead to a number of interesting new starches, which have helped to shape the scientific knowledge about starch biosynthesis in general.

By altering the starch structure, granular starches might be obtained with novel physical properties and a potentially unlimited range of new industrial applications. This has proven to be more difficult although altered morphology of starch granules has been obtained in potato plants in which the activity of two specific starch synthases has been inhibited or in plants in which in starch granules co-polymers of starch and fructan were produced.

Analysis of these starches from several transgenic plants with scanning electron microscopy demonstrates severely altered granule morphology. In addition, the granule size distribution is significantly modified. We have analyzed in detail the level and time point during tuber development of the occurrence of altered granules. This knowledge is used to isolate RNA of the crucial developmental stage for further analysis using a potato oligo micro array.

DEVELOPING THE POTATO BIOTECH VARIETIES WITH THE IMPROVED AGROTECHNICAL PROPERTIES.

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Three Russian potato varieties were transformed via *A. tumefaciens* strain with *cryIIIa*/*CP4* construct. The field trials for selected lines were performed at 3 Russian regions. Lines were tested for their resistance to Colorado potato beetle (CPB) and agronomic performance. Further the detailed molecular analysis (including the integrity of inserts, insert copy number estimation and unique border identifier search) for selected in field trials lines was made. In whole, 17 lines of GM Lugovskoi, 10 lines of GM Nevski and 2 lines of GM Elizaveta were tested and 4, 2 and 2 single-copied between them were put in the process of commercialization. All candidate lines were applied for Russian Seed Committee approval – varieties registration. Also all candidate lines with their unique border identifiers were applied for patenting. The food tests for the most advanced lines (candidates for commercialization) for 2 GM varieties (Elizaveta Plus and Lugovskoi Plus) had been finished with the obtaining the State Food Safety Certificate (signed by Major Sanitary Inspector of Russian Federation).

Russia is the third potato breeding country in the world. The calculated demand for potato in Russia is 122-125 kg per person per year or 30-35 billion tons of the total harvest.

At nowadays level of potato breeding potential harvest losses are valued by Russian academy of Agriculture experts up to 4.1 billion tons per year on the amount of 19.4 billion rubles. Besides as more than 95% of potato production is concentrated in privet sector where without proper measures the losses are estimated from 40% to 80% and real annual losses because of Colorado potato beetle are 2-2.2 trillion dollars.

That was the reason of starting the "Russian Bt-potato project". The actuality of the project fulfillment is determent by the necessity of Food safe state program. The goal of the project is to develop effective seed production system. This system is to be founded on the base of obtained Russian biotechnological potato varieties resistant to Colorado potato beetle. The distribution system will be part of project. This project is absolutely unique for agricultural and biotechnological Russian market and is supposed to be financed from commercial sources to obtained successful realization.

On the first stage of the project three Russian potato varieties were transformed via *A. tumefaciens* strain with Bt/CP4 construct. At least 100 transformed plants (individual transformation events) were obtained for each variety. All transformed plants were assayed for Bt gene expression levels. Lines with more than 10 ppm level of expression were selected for further work.

The second stage of the project was devoted to field trials. The field trials for selected lines were performed at 3 Russian regions. All selected at 1st stage lines were tested for their resistance to Colorado potato beetle (CPB) and agronomic performance.

The experiments were made according to following program:

- Transgene efficacy
- The crop and typeness productivity.
- The comparative study of susceptibility to phytophatogenes (including fungi, viruses and bacteria) for transgenic and control varieties.
- The study of the resistance to herbicide (if necessary to destroy transgenic plants).
- The study of the applicability of the traditional potato breeding schemes for transgenic varieties.
- The reproduction of new property (resistance to beetle) along the vegetative generations.
- The possible influence of transgenic plants on soil microorganisms.
- The study of the influence of the transgenic plants on the target and non-target organisms (insects).
- Resistance management studies.

- The seed pool increase.

Further the detailed molecular analysis (including the integrity of inserts, insert copy number estimation and unique border identifier search) for selected in field trials lines was done in parallel with field testing.

In whole, 17 lines of Bt/CP4 Lugovskoi, 10 lines of Bt/CP4 Nevski and 2 lines of Bt/CP4 Elizaveta were tested and 4, 2 and 2 single-copied between them were put in the process of commercialization (one major candidate line for commerce and back up ones).

All candidate lines were applied for Russian Seed Committee approval – varieties registration (priority dates – 2004/09/11). Also all candidate lines with their unique border identifiers were applied for patenting (priority dates – for Elizaveta line 2004/23/11; for Nevski line 2005/14/02)

The food tests for the most advanced lines (candidates for commercialization) for 2 BT/CP4 varieties (Elizaveta Plus and Lugovskoi Plus) had been finished with the obtaining the State Food Safety Certificate (signed by Major Sanitary Inspector of Russian Federation).

Thus, all Research and Developing work is done. All the next activities are to be devoted to the regulation process and seed production.

AGROBACTERIUM - MEDIATED TRANSFORMATION OF *S. BULBOCASTANUM* AND POTATO (*S. TUBEROSUM* CV. DELIKAT) WITH MSH2 DEFFICIENT GENES

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Somatic hybridization between *Solanum tuberosum* and *S. bulbocastanum* could be a very useful strategy in order to obtain new potato cultivars with enhanced resistance to late blight (*Phytophthora infestans*). The useful trait transfer can be enhanced by inducing a mismatch repair sistem deficiency in one of the two partners by genetic transformation.

S. bulbocastanum although well established as highly resistant to late blight, is a very recalcitrant wild species to “in vitro” regeneration. Therefore, prior to genetic transformation experiments a successful and reliable shoot regeneration protocol is to be established to induce organogenesis. Our studies revealed positive results in inducing *S. bulbocastanum* to regenerate callus by adding the phytohormones IAA or 2,4-D to the regeneration medium, instead of NAA which proved to work for other *Solanum* species.

Also in this study *Agrobacterium tumefaciens* - mediated transformation of *Solanum tuberosum* cv. Delikat with altered mismatch repair system genetic constructs FRG-MSH2-Apa and FRG-MSH2-As (both representing 2 types of *msh2* deficient gene) was performed, in order to induce enhanced variability in novel genetic recombination during subsequent somatic hybridization with *S. bulbocastanum*.

In the case of *S. tuberosum* cv. Delikat, the addition of AgNO3 induced shoot biomass accumulation in both controls or transformed lines. Shoot regeneration in the presence of *Agrobacterium tumefaciens* was induced for both genetic constructs used in this study.

IMPROOVING CROP GENETIC POOL BY SOMATIC HYBRIDIZATION USING DNA MISMATCH REPAIR (MMR) DEFFICIENT PLANTS

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The goal of this study was to recover a high number of hybrid plants resulted from protoplast fusion between *S. tuberosum* and *S. chacoense*. The novelty of the method consists in using as fusion partner for potato the MMR deficient transgenic *S. chacoense*. The mutated protein, MSH2, plays a key role in the MMR system in plants.

Transgenic plants of *S. chacoense* were obtained by *Agrobacterium*-mediated transformation. Two strains of *Agrobacterium* were used carrying different plasmids: FRG-MSH2-As (antisens = *As*) and FRG-MSH2-Apa (mutated form = *Apa)*. Protoplasts of diploid wild type *S. chacoense* and MMR deficient plants were electrofused with tetraploid *S. tuberosum* cv. Delikat. The plating efficiency and the number of plants recovered from each fusion combination were followed. The ploidy of putative somatic hybrids was determined by flow cytometry. Somatic hybrid clones were tested for resistance to Colorado potato beetle.

The plating efficiency was better for the combination *S. tuberosum* + *S. ch. Wt* (21.7% compared to 10.8% for *Apa* and 5.6% for *As*). The number of regenerated putative hybrid plants was higher *for S. tuberosum* + *S. ch. Apa* (162 plants) or *As* (213 plants) compared to *Wt* (13 plants). Out of 72 analyzed plants 39 were hexaploid or mixoploid somatic hybrids.

The regeneration of a high number of hybrid plants opens the way for transferring resistance genes, for instance to Colorado potato beetle into potato gene pool.

The deficiency of MMR is expected to overpass the possible mismatches of the base pairs and to induce a high frequency of homeologous recombination during mitosis and meiosis.

MICROTUBERIZATION AS AN EFFICIENT WAY FOR *IN VITRO* MEDIUM-TERM CONSERVATION OF *SOLANUM* WILD SPECIES, POTATO SOMATIC HYBRIDS OR TRANSGENIC PLANTS

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Breeding new potato varieties through biotechnological means is today one of the main goals of potato breeding. Traits such as resistance to diseases (*Phytophthora*, viruses) or pests (Colorado potato beetle), can be transferred and integrated into potato gene pool either by protoplast fusion derived somatic hybrids or by the transfer and integration of resistance genes, isolated from other organisms, into cultivated potato.

The new biotechnological approaches are addressing either pre-breeding for new varieties or the conservation of valuable material as micro tubers *in vitro*. By inducing microtuber development *in vitro* better micropropagation of true to type lines as well as conservation of valuable genetic resources were achieved. Tuberization is a complex process, resulting in stolon differentiation into tubers. Sucrose metabolism is modified during tuber formation, but many other factors including phytohormones are playing a part into this complex process. We tried to induce tuberization without using phytohormones because their role in inducing genetic variability is well known. Different concentration of sucrose (6%, 8%, 10%) in MS media (Murashige and Skoog, 1962), were used to cultivate nodal fragments and induce microtuberization in *Solanum tuberosum* cv. Désirée and in the wild specie *Solanum microdontum*. The explants were cultivated at 21°C and a photoperiod of 16h/8h. The experiments were carried out during a whole year, but the microtubers lasts *in vitro* longer. For tuberization in cv. Désirée the best sucrose concentration was 10% and in *Solanum microdontum* the best response was for 8% sucrose into MS medium. Afterwards, the tuberization was successfully induced by 10% sucrose in putative fusion products between *Solanum chacoense* MSH2 defficient mutants and two potato cultivars: *Solanum tuberosum* cv. Delikat and *Solanum tuberosum* cv. Désirée.

Microtubers proved to be a very suited material for medium-term conservation of potato valuable genetic resources.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

VARIETAL ASSESSMENT

THE POTATO VARIETIES IN VARIOUS SOIL AND WEATHER CONDITIONS

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INTRODUCTION

Although potato is quite adaptable to planting conditions and it is considered to be cultivatable in almost every climate and soil conditions, it has, as every culture, certain requirements for ecological conditions. Stability of a varieties features and preserving quality in different conditions has great importance; total and graded yield and quality factors are affected by variety and location (Crop Monitor, 2003, Tomasiewicz et al., 2003, Haase et al., 2005).

Every potato producer is aware that locally bred varieties are better to cultivate in local conditions. But there conditions might also have different soil or weather conditions. Tuber yield and quality might be affected by the length of vegetation period and weather, soil type and soil texture. The weather conditions that affect yield the most are precipitation and air temperature (http://www.gvc.gu.se/… 2002, http://mars.jrc.it/marsstat/Crop_Yield_Forecasting…,)

Being a part of the European Union (EU) it is allowed to import every potato variety on a list of the varieties of the EU. But the practice has shown that the varieties do not act according to their description in the countries far from the origin of a variety. To provide the scientific explanation of that kind of action the trials were carried out at different location in Estonia. The results should explain the previously mentioned situation of practice.

MATERIALS AND METHODS

The research observed four varieties of Jõgeva Plant Breeding Institute: 'Anti' (late), 'Maret' (medium early), 'Piret' (medium), 'Reet' (early to medium) and one breed 1182-97 (medium late). The trials were carried out at three different locations in Estonia: at the Jõgeva Plant Breeding Institute (Jõgeva), at the Kuusiku Testing Centre of Agricultural Research Centre (Kuusiku) and in the experimental field of the Agricultural and Environmental Institute of Estonian University of Life Sciences (Tartu) in 2005. The seed potato originated from Jõgeva Plant Breeding Institute. All the fields had similar cultivation and fertilization. The testing field of **Jõgeva** was located on medium sandy loam *Calcaric Luvisol* soil, **Tartu** experimental field was located on medium sandy loam *Stagnic Luvisol* soil. The testing field of **Kuusiku** was located on light loamy *Rendzic Leptosol* soil (Reintam, Köster, 2006). The analysis of the yield (tuber yield, weight of tuber and tubers per plant), starch content and culinary traits (taste, mealiness) of every testing place was carried out at Jõgeva Plant Breeding Institute (Jõgeva PBI).

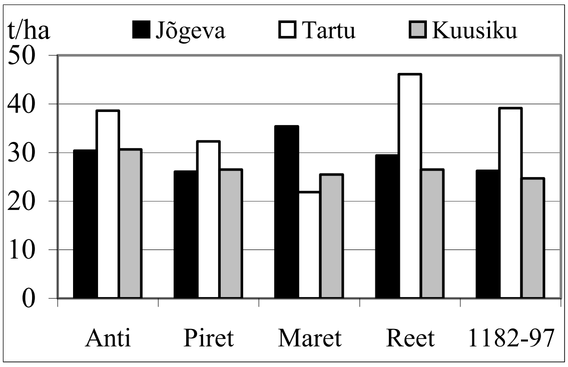
As certain affective factor in potato yield and quality is precipitation during the vegetation period, the table of precipitation amounts at different locations is brought out in Table 1. Potato needs 2,5-3 mm of precipitation per day in June and 4-6 mm in July. At Jõgeva there was an average precipitation 1,7 mm per day in June and 1,9 mm in July and at Kuusiku 2,6 mm in June and 1,6 mm in July. Warmer than average was in July, but at the same time there wasn't enough moisture in soil for potato. The productive water supply in the beginning of July was at lower than optimal level was. If the need of water during the active growth period would be 300 mm of precipitation, then according to references they may be divided as follows: 70 mm in June, 120 mm in July and 90 mm in August. But at Jõgeva it was 50 mm and at Kuusiku 79 mm in June, in July accordingly 60 mm and 50 mm, in August 116 mm and 171 mm. Precipitation was lowest in July (25 mm) and August (96 mm) at Tartu.

Table 1. Precipitation (mm) during the vegetation period in 2005

Location/Month	June	July	August
Norm loamy/sandy soil	70/85	120/150	90/115
Jõgeva	50	60	116
Kuusiku	79	50	171
Tartu	69	25	96

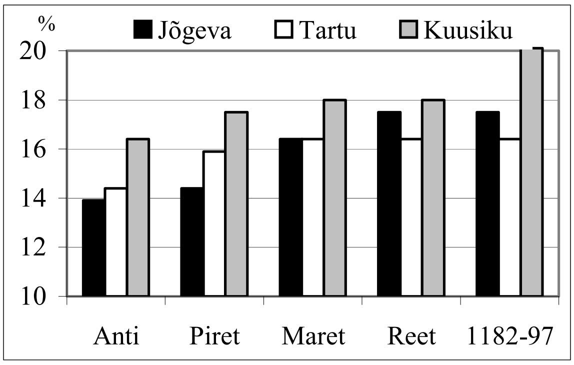
RESULTS AND DISCUSSIONS

Tuber yield. Figure 1 shows that the early variety 'Maret' and medium variety 'Piret' had relatively low yield, because there was precipitation under the norm in June and July and due to drought the plants couldn't absorb the nutrients. The highest yield had 'Maret' at Jõgeva, because it formed earlier before the drought period and was affected by the precipitation at the end of July. In July there was the least of precipitation at Tartu and probably that affected the yield of 'Maret' so much that it appeared to be significant the lowest of three locations. The yield of 'Piret' was the highest at Tartu. There was no significant difference in yields at Jõgeva and Kuusiku. The variety 'Reet' had the highest yield at Tartu, which was probably caused by favourable soil and precipitation in August. 'Reet has significant difference in yields at Jõgeva and Kuusiku, although the amount of precipitation was almost the same, but the gravely soil at Kuusiku is more pervious. The late variety 'Anti' could use precipitation in August, which was over the norm in all three locations (a bit less at Tartu). The yield of 'Anti' at Jõgeva and Kuusiku were practically equal, significant higher (38,6 t/ha) in Tartu, probably caused by favourable soil and weather conditions. The yield of medium late breed 1182-97 was also the highest at Tartu. The reasons were the same as had 'Anti'.



$LSD_{05} = 9,8$

Figure 1. Tuber yield (t/ha) in various locations

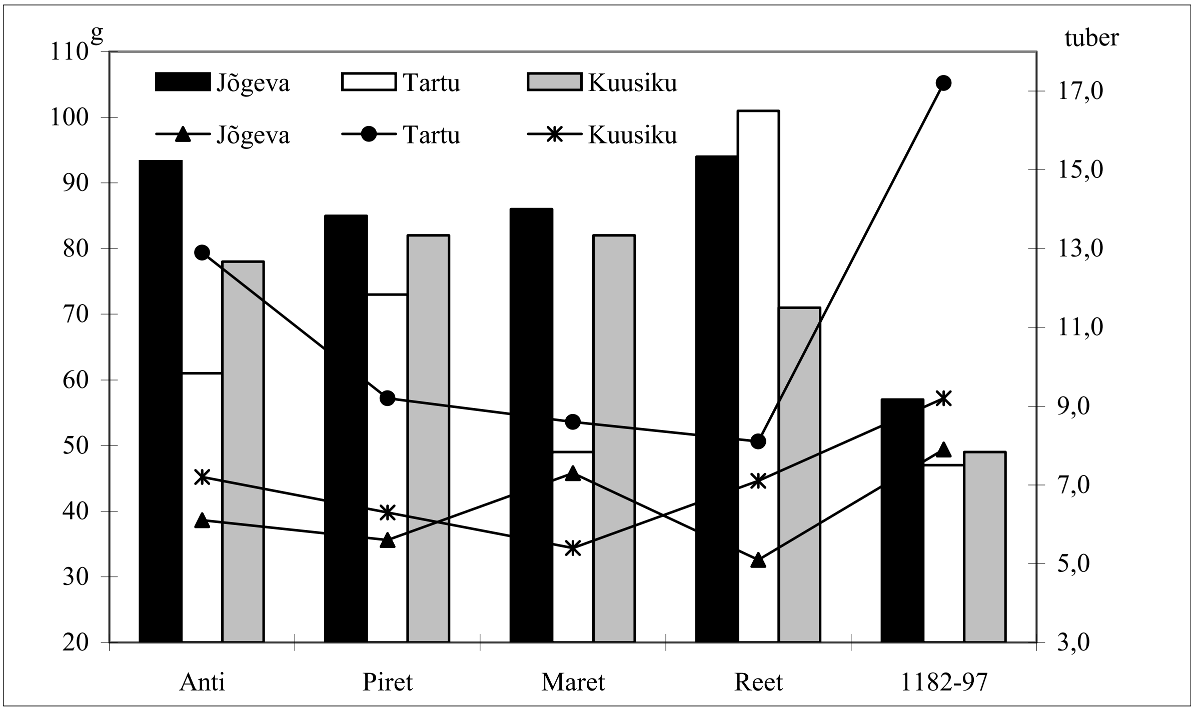


$LSD_{05} = 1,1$

Figure 2. Starch content (%) in various locations

Starch content of tubers. Figure 2 shows the differences of starch content of tubers depending on variety and location. Significant the highest starch content was at Kuusiku, although there was more precipitation at the active vegetation period than at Jõgeva and Tartu. But the gravely soil at Kuusiku is more pervious and suffered more under the drought. Warm and sunny weather increases starch content of tubers. The summer of 2005 was especially droughty at tested locations. The varieties 'Anti' and 'Maret' had no significant differences in starch content at Jõgeva and Tartu. The starch content of the variety 'Anti' ranged from 14,0 % to 16,5% and of the breed 1182-97 from 16,3% to 20,0% in different locations.

Tuber weight and tubers per plant. Tuber weight and the number of tubers per plant are closely related (Figure 3). Most of the tested varieties and the breed at Jõgeva had the biggest average weight of tuber and the smallest in Tartu. There was also the smallest number of tubers per plant at Jõgeva and the biggest at Tartu (Figure 3). That was caused by drought at Jõgeva in June, due to what only few tubers formed per plant, but later the precipitation at the end of July and in August increased these tuber weight. But at Tartu, the amount of precipitation was average, so the formation of tubers was possible and the drought in July caused the small weight of tuber. The smallest average weight of tuber had the variety 'Maret' and the breed 1182-97 at Tartu. Tuber growth of the early variety 'Maret' was hampered by drought therefore it couldn't form more tubers. Medium late breed 1182-97 grew fast at the beginning and had a longer period of growth and even the late variety 'Anti' (slow primary growth), but could not grow big enough due to drought. Significant difference of tuber weight didn't appear or it was minimal at Jõgeva and Kuusiku, the same tendency appeared about the number of tubers per plant.



$LSD_{05\ \text{weight of tuber}} = 6{,}0$; $LSD_{05\ \text{tubers per plant}} = 0{,}9$
Tuber weight as column, tubers per plant as line

Figure 3. Tuber weight (g) and tubers per plant (tuber) in various locations

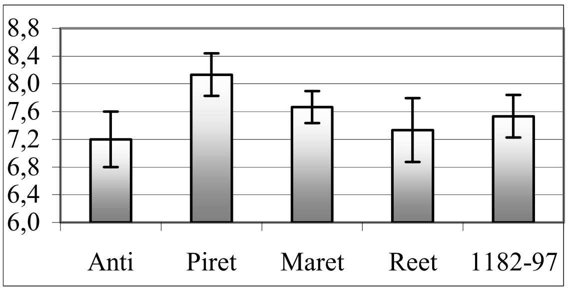


Figure 4. Taste (1-9 points, 9-excellent) in various locations

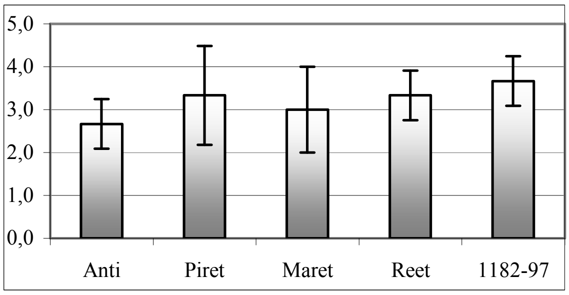


Figure 5. Mealiness (1-5 points, 5-dry and very mealy) in various locations

Taste and mealiness. The taste is not so important in potato breeding at the moment, but as our consumers use potato as table potato we also estimate the taste. The varieties 'Ants' and 'Reet' differed more by taste in various locations. Little bit worse taste had varieties at Tartu, except 'Piret'. The best tasting potato grew on gravely soil at Kuusiku. As likely it resulted from gravely soil in Kuusiku (Figure 4)

By long-time analysis of trials can conclude that mealiness of cooked potatoes is to large extent dependent on starch content, which in turn depend on the variety and weather. Trial analysis showed that mealiness of the varieties 'Piret' and 'Maret' varied the most of all (Figure 5).

The variety 'Piret' varied most (3%) within the range of the starch content in various locations. The starch content and mealiness were much higher at Kuusiku. Taste and mealiness were only lower in variety 'Anti' because it ended the growth by early night frost and tubers could not ripe. The gravely soil of Kuusiku suffered by drought more and therefore was accumulated more starch. Varieties

‘Maret’, ‘Reet’ and breed 1182-97 were less mealy at Tartu.

SUMMARY

1. Tuber yield. Significant the highest tuber yield grew at Tartu, except the variety ’Maret’. As in Tartu there was the least of precipitation during the vegetation period (190 mm) and in Kuusiku the most (300 mm) the highest yield at Tartu was affected by better moisture regime caused by bigger water capacity of the soil. The varieties ‘Anti’, ‘Piret’ and breed 1182-97 at Kuusiku and Jõgeva trials had no significant differences in yield.

2. Tuber weight and number of tubers per plant. Most of tested varieties and breed at Jõgeva had credibly the biggest average weight of tuber, except the variety ‘Reet’, the smallest at Tartu. Though at Jõgeva there was the smallest number of tubers per plant and at Tartu it was the biggest. The cause of it was the drought at Jõgeva in June and beginning of July. At Tartu the amount of precipitation was normal in June during the formation of tubers, but because of drought in July and August the tubers grew smaller. There was no significant difference among average weight of tuber, or it was minimal, at Jõgeva and Kuusiku. The same tendency appeared about the number of tubers per plant.

3. Starch content of tubers. Significant the highest starch content was at Kuusiku. Having the highest amount of precipitation during the growth period, but also having more pervious soil, the deficiency of water appeared and that caused formation of high starch content.

4. Taste and mealiness of same variety differed in various locations.

As a result of the tests it is possible to state that the factors of yield and quality of the same variety may vary in different locations. If these differences appear even in such small country as Estonia is, then it is true that the varieties do not act according to their description in the countries far from the origin of a variety.

Acknowledgements

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EVALUATION OF YIELD PERFORMANCES OF SOME POTATO CULTIVARS FROM DIFFERENT MATURITY GROUPS IN A MEDITERRANEAN-TYPE ENVIRONMENT IN TURKEY

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INTRODUCTION

Potato is grown as early crop during winter and spring months in Mediterranean type environments in Turkey (Caliskan et al., 1997). This cropping system is characterized with shorter growing period (80-100 days), short photoperiod (10-12 hours), and high day temperatures (>28 C) during tuber bulking stage. The potato cultivars bred in North European countries are commonly used in both main crop and early crop production in Turkey. However, the potato growing conditions in Mediterranean region are opposite of potato growing conditions in North European countries. The short photoperiod accelerates tuber initiation, and results in less vegetative development whereas the high temperature stress limits tuber bulking after mid April. Therefore, selection or improvement of appropriate cultivars for early production in Mediterranean conditions is very important (Foti, 1999; Frusciante et al., 1999; Caliskan et al., 1999; Caliskan, 2001). There is also a tendency to recommend early genotypes for early potato production in Mediterranean type environments due to short growing period. However, the cultivars can not express their characteristics in most times due to stressful environment of Mediterranean region. Therefore, specific adaptability of potato cultivars to early potato production conditions of Mediterranean region is more efficient on their yield and quality performances than their maturity groups (Caliskan, 2001). Previous findings often indicated that later maturing genotypes gave the higher yield values than very early or early cultivars. A number of cultivars have been introduced to Turkey in each year. The evaluation of new cultivars under Mediterranean conditions is very important to select most suitable cultivars for early potato production. In this study, the yield performances of sixty five potato cultivars from different maturity groups were evaluated in a Mediterranean-type environment in Turkey during 2000 and 2004.

MATERIAL AND METHOD

This study was conducted at the Experimental Farm of Agricultural Faculty, Mustafa Kemal University in Hatay, Turkey (36^o 15’ N, 36^o 30’ E) during winter and spring seasons between 2000 and 2004. The soil of the experimental fields has low organic matter content and was slightly alkaline in reaction. Hatay province has typical Mediterranean climate conditions with hot-dry summers and mild-rainy winters.

The number of cultivars varied in years, but sixty five potato cultivars from different maturity groups were evaluated in total during five year period. The name and maturity groups of the evaluated cultivars as well as planting and harvesting dates in the experimental years were presented in Table 1. The experiments were laid out in randomised complete block design with three replications in each year. Medium size (35-55 mm) seed tubers of cultivars were planted with 70 and 25 cm inter- and intra-row spacing using a feed assisted planter. Plots were fertilized with 90 kg N, P, K per ha using a composed fertilizer before planting, and an additional nitrogen dose of 60 kg per ha as ammonium nitrate was side-dressed at the beginning of tuber bulking. Standard cultural practices were employed to the plots during growing periods.

RESULTS AND DISCUSSION

The tuber yield values of evaluated cultivars between 2000 and 2004 were presented in Table 1, and overall yield performance of different maturity groups during five-year period was given in Table 2.

Table 1. Yield performance of potato cultivars evaluated between 2000 and 2004 in Hatay, Turkey.

Cultivars	Maturity Group	Tuber Yield (t ha^{-1})				
		2000	2001	2002	2003	2004
Adora	Very early	18.5	--	--	22.5	--
Alaska	Very early	--	--	--	--	29.8
Anais	Very early	--	--	--	--	26.6
Binella	Very early	--	--	--	33.7	--
Concorde	Very early	24.0	--	--	--	--
Elodie	Very early	--	--	--	--	16.7
Ilona	Very early	15.0	--	--	--	--
Isabel	Very early	--	--	--	30.0	--
Jaerla	Very early	15.0	20.9	29.7	--	--
Justine	Very early	--	--	--	--	21.5
Minerva	Very early	--	--	--	20.4	
Safrane	Very early	--	--	--	--	24.4
Velox	Very early	--	30.3	35.5	--	--
Winston	Very early	--	--		15.6	--
Accent	Early	--	28.9	21.4	--	--
Agata	Early	18.0	--	--	--	--
Arnova	Early	--	36.8	--	--	--
Carlita	Early	19.0	--	--	--	--
Concurrent	Early	25.1	--	--	--	--
Crona	Early	--	--	--	--	18.2
Eve Balfour	Early	--	--	--	18.5	--
Impala	Early	23.0	--	--	--	--
Latona	Early	26.9	--	-	--	--
Marabel	Early	25.0	35.2	34.9	25.9	24.8
Tomensa	Early	--	25.2	--	--	--
Ausonia	Medium early	20.4	--	--	--	--
Cycloon	Medium early	--	27.7	--	--	--
Fabula	Medium early	16.7	--	--	--	--
Felsina	Medium early	22.9	--	--	--	--
Goliath	Medium early	--	--	--	--	21.3
Konsul	Medium early	--	27.8	--	--	--
Hermes	Medium early	--	22.9	--	--	--
Lilla	Medium early	--	--	--	--	36.6
Maranca	Medium early	--	31.2	--	--	--
Marfona	Medium early	22.4	27.0	25.3	22.3	19.5
Monalisa	Medium early	25.5	21.7	21.7	--	--
Provento	Medium early	--	33.9	--	--	--
Satina	Medium early	24.8	--	--	--	--
Shepody	Medium early	13.8	13.0	--	--	--
Solide	Medium early	--	34.5	15.0	--	--
Spunta	Medium early	--	--	--	31.5	--
Victoria	Medium early	21.1	--	--	--	--
Vivaldi	Medium early	16.1	--	--	--	--
Doline	Medium	--	--	--	--	23.1
Harmony	Medium	--	--	--	22.9	26.7
Hopehely	Medium	--	--	--	--	11.0
Sebastian	Medium	--	--	--	--	28.6
Szazszorszep	Medium	--	--	--	--	20.3
White Lady	Medium	--	--	--	--	23.3

Table 1. continued.

Cultivars	Maturity Group	Tuber Yield (t ha^{-1})				
		2000	2001	2002	2003	2004
Agria	Medium late	20.5	30.9	20.2	19.8	20.0
Argos	Medium late	--	--	--	--	27.7
Buchan	Medium late	--	--	--	33.1	24.0
Granola	Medium late	21.0	--	23.7	--	--
Lady Balfour	Medium late	--	--	--	27.9	--
Marena	Medium late		36.6			
Mondial	Medium late	18.7				
Morene	Medium late	25.4				
Remarka	Medium late	24.5				
Sante	Medium late		27.9			
Van Gogh	Medium late	27.0	38.0	31.6	32.9	24.4
Cara	Late			24.1	23.6	
Donella	Late	10.2				
Kankan	Late					11.3
Russet Burbank	Late	13.9	15.1			
Slaney	Late	--			24.2	
Mean		20.7	28.3	25.7	24.7	22.8
LSD (%5)		1.3	2.9	2.1	2.8	1.9
CV(%)		3.8	6.3	4.7	6.8	5.0
Planting date		Mar 9	Feb 28	Jan 19	Jan 22	Mar 1
Harvesting date		Jun 15	Jun 23	May 17	Jun 11	Jun 19

The evaluated cultivars showed significant differences for total tuber yield in each experimental year. Tuber yield values ranged from 10.2 t/ha (Donella) to 27.0 t/ha (Van Gogh) while the mean tuber yield was 20.7 t/ha in 2000 (Table 1). Late cultivars and very early cultivars (ecept Concorde) yielded less than experimental mean in 2000. The cultivars from maturity groups of early, medium early, medium or medium late were generally yielded more than experimental mean although some cultivars from each maturity group produced considerably lower tuber yield than the overall mean.

Twenty cultivars from five maturity groups were evaluated in 2001 (Table 1). The average tuber yield was the highest (28.3 t/ha) in 2001 among the five experimental years. Medium late cultivars Van Gogh (38.0 t/ha) and Marena (36.6 t/ha), and early cultivars Arnova (36.8 t/ha) and Marabel (35.2 t/ha) were distinguished as the highest yielding varieties in 2001. The lowest tuber yields were obtained from medium early cultivar Shepody (13.0 t/ha) and from late cultivar Russet Burbank (15.1 t/ha) in this year.

Very early cultivar Velox produced the highest tuber yield (35.5 t/ha) and followed by an early cultivar Marabel (34.9 t/ha) in 2002. Similarly, a very early variety Binella was also gave the highest tuber yield (33.7 t/ha) in 2003 whereas the lowest tuber yield was obtained from another very early cultivar Winston (15.6 t/ha) in this year. Medium late cultivars Buchan (33.1 t/ha) and Van Gogh (32.9 t/ha), and medium early Spunta were also classified within the highest yielding cultivars in 2003. It was interesting to notice that the highest yields were obtained from very early cultivars in 2002 and 2003 which the earliest plantings was realized in these years among the five experimental years.

Twenty one cultivars from six maturity groups were evaluated in 2004 (Table 1). Tuber yield values varied between 11.0 t/ha (Hopehely) to 36.6 t/ha (Lilla) while the mean tuber yield was 22.8 t/ha in 2004. There was only one cultivar from late maturity group (Kankan) and this variety also showed very poor yield performance in this year. However, we could not classified other maturity groups as high yielding or low yielding since there were both high and low yielding cultivars in each group.

Table 2. Overall yield performance of different maturity groups in Hatay situated in East Mediterranean Region in Turkey.

Maturity Group	Number of Cultivars	Mean Tuber Yield (t ha^{-1})	Tuber Yield Range (t ha^{-1})
Very Early	14	23.9	15.0-35.5
Early	11	25.4	18.0-36.8
Medium Early	18	23.7	13.0-36.6
Medium	6	22.3	11.0-28.6
Medium Late	11	26.5	18.7-36.6
Late	5	17.5	10.2-24.2

It may difficult to derive definite conclusions about maturity group performances from this study since the number of cultivars from each maturity groups varied among years. However, we can derive some presumptions from our results. There were not significant differences between maturity group means except late group (Table 2). We can conclude that late maturing cultivars were much affected by high temperature stress occurred after May since their crop growth and tuber growth rates are slower than earlier ones in general. This indicates that late maturing cultivars may not suitable for early potato production under Mediterranean conditions. However, we can not definite conclusions about other maturity groups since there were found very high yielding cultivars in each maturity group. It can be concluded that the cultivars, which are better tolerate to environmental stresses peculiar to Mediterranean climate such as short photoperiod and high temperature, should be selected and cultivated in early potato production in Mediterranean-type environments. Hence, adaptation studies with introduced or improved cultivars should be conducted continuously under Mediterranean conditions.

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STABILITY OF RESISTANCE TO *PHYTOPHTHORA INFESTANS* IN POTATO CULTIVARS EVALUATED IN THE FIELD AND LABORATORY EXPERIMENTS.

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Stability of resistance across locations and over time is difficult to study because it requires historical data so that inferences about stability may be drawn over a significant period. A genotype is considered to be stable resistant, if among-environment variance of resistance is small. Becker and Léon (1988) referred to this stability as a static or biological. A stable genotype possesses an unchanged performance regardless of any variation of the environmental conditions. Analyses of genotype by environment interactions and estimation of biological stability have been studied during the last decades and several methods were proposed for its estimation.

In this study, genotype by environment (G × E) interactions and biological stability of resistance to *Phytophthora infestans* were analyzed for 22 potato cultivars, using multi and one-dimensional statistical methods. The Sergen program developed by Caliński et. al. (1998) was applied for these analyses. The potato cultivars were tested in four experiments in Central and Southeastern parts of Poland over four years.

The cultivars were planted at each location at the beginning of April and were cultivated without protection against late blight. Tested cultivars were exposed to the natural infection with *P. infestans*. Each cultivar was grown on 6-hill plots in two replications surrounded by susceptible cultivar, which served as infector. The development of late blight in foliage was evaluated weekly using 9 grade scale (where 9 = resistant). The relative area under the disease progress curve (rAUDPC) for each cultivar was calculated after field observations.

The analysis of variance of diseases data (rAUDPC) for 22 cultivars in 13 environments indicated significant ($P<0,01$) influence of genotype (G), years (Y), environments (E) and interaction G × E. The applying multidimensional statistical revealed that the most stable cultivars were Bzura, Wawrzyn, Meduza (high resistance) and Klepa, Jasia, Nimfy, Ania, Hinga, Vistula (medium resistance). These cultivars had stable level of resistance to *P. infestans* at four locations during four cropping seasons. For stable cultivars interaction with years, locations and environments were not significant. Moreover, for these cultivars coefficient of regression and deviation from regression were not significant.

In a group of unstable cultivars, interaction G × E and deviations from regression were significant. Unstable cultivars were divided into two groups: "regularly unstable" (intensity of infection corresponded to infection pressure) and "unstable with unpredictable reaction".

The study of G × E interaction structure using AMMI model (additive main effects and multiplicative interaction) revealed what was the contribution of each cultivar to this interaction. Cultivars, which expressed unstable level of resistance, participated significantly in this interaction. Moreover, the study of G × E interaction structure showed also that one location with high infection pressure had an important contribution to this interaction.

Variable rainfall and temperature conditions may affect the stability of resistance to late blight or expression of resistance. The applying of AMMI model enabled to reveal potato cultivars with resistance to late blight, which was stable in successive years, different locations and environments. Such cultivars can be recommended for cultivation in various environments. A differential response of potato cultivars to environmental factors suggests that it is advisable to evaluate resistance of potato cultivars in various locations for several years.

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RESULTS OBTAINED TO SOME POTATO VARIETIES UNDER THE SPECIFIC CONDITIONS FROM SOUTH ROMANIA

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Abstract

Potato growers have to know from different points of view the potato varieties (morphological and physiological characteristics, productivity and yield quality, agronomic characteristics, resistance to diseases and pest as well as to other stress factors) in view to correctly choose the varieties they will cultivate as to provide them with high and qualitative yields. This is more necessary as there are some potato varieties on the local markets that are less known or even unknown by the potato growers. In the present paper there are presented some of results obtained concerning the foreign potato varieties admitted to be cultivated in Romania and studies under the climatic conditions of the years 2006 and 2007 in the specific conditions from South Romania. The studied varieties were the following: Velox, Fabula, Carrera, Sprint, Rosara, Lady Claire and Lady Rosetta.

Key words: Potato; Varieties; South Romania.

Introduction

The assortment of potato varieties admitted to be cultivated in Romania increased very much in the last years by breeding new potato varieties, and also by cultivating some new varieties from abroad which are admitted to be cultivated in Romania according to the actual regulations. In the last years, numerous foreign potato varieties were added to the list of Romanian varieties, which were already registered in the Romanian Official Catalogue. To these foreign potato varieties already registered into the Official Catalogue of crop varieties grown in Romania, there are added the potato varieties registered into the National Official Catalogue of one of the European Union countries, which could be cultivated in Romania according to the law. Thus, the assortment of potato varieties currently accepted for cultivation in Romania has become extremely diverse, in the Official Catalogue of crop varieties grown in Romania for the year 2006, there been 83 registered potato varieties, among which 24 are foreign varieties [1].

The foreign potato varieties, but especially the ones that are not registered into the Official Catalogue of crop varieties grown in Romania but admitted to be cultivated because they are registered into one of the European Union countries are less known or even unknown with respect to the physiological and productive characteristics, and resistance to diseases and pest as well as to other stress factors. But, the potato growers have to know all these varieties regarding their characteristics in view to correctly choose the variety according to their specific growing conditions.

Materials and Methods

Researches were carried out in field experiments located 15 km Northeastern faraway from Bucharest, in the years 2006 and 2007. The field experiments were located within the experimental farm of the Bucharest University of Agronomical Sciences and Veterinary Medicine – Faculty of Agriculture.

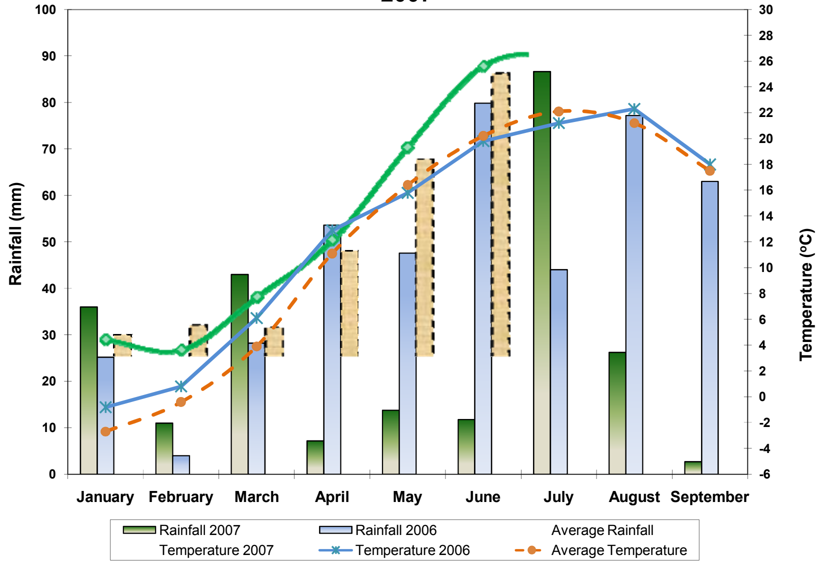
The studied varieties were the following: Velox (yellow tubers), Fabula (light yellow tubers), Carrera (yellow tubers), Sprint (white tubers), Rosara (red tubers), Lady Claire (white to yellow tubers, indicated for chipping) and Lady Rosetta (red tubers, indicated for chipping).

The field experiments had random plots with four replications. Each plot had a surface of 60 m^2, which meant eight plant rows grown at a distance of 75 cm between rows and 10 m distance along the rows. The experiments were drip irrigated and the planting density was 53,300 tubers per hectare, except the two varieties indicated for chipping for which the density was 49,300 tubers per hectare.

The research objectives were to study the potato varieties from a productive point of view and to observe some specific characteristics that can be useful for the potato grower. The determinations were focused on the following: average number of tubers per plant, average weight of tubers (g), maximum weight of tubers (g), yield (t/ha), number of days from emergency to maturity, and some specific characteristics observed during growth period.

In the year 2006 (figure 1), the average air temperature was higher than the annually average on spring (except May), August and September, and was smaller than the annually average on May, June and July, which means that in the active growth period for potato the temperature was more favorable than in other years. But, in the year 2007, the average air temperature was much higher than the annually average all over the year. Concerning the rainfall, in the year 2006, the monthly rainfall sum were higher than the annually average on April, August, and September, but in total there was a deficit of 20,4 mm for the first nine months compared to the annually average. But, in the year 2007, the monthly rainfall sum were higher than the annually average on January, March and July, and much smaller in the rest of the year, thus in total there was a deficit of 204,7 mm for the first nine months compared to the annually average.

Figure 1. Rainfall and temperature in the years 2006 and 2007



Results and Discussions

The average number of tubers per plant (table 1) in the two experimental years (2006 and 2007) varied between 6 (Fabula and Sprint varieties) and 14 (Lady Claire and Lady Rosetta).

The average weight of tubers varied in the two experimental years from 61 g (Lady Claire variety) to 188 g (Fabula variety). High value of the average weight of tubers was realized also at the Sprint variety (160 g) and Carrera variety (126 g).

Potato varieties very good for chipping (Lady Claire and Lady Rosetta) realized the highest number of tubers per plant (14), but the smallest weight of tubers, respectively 61 g for Lady Claire variety and 63 g for Lady Rosetta variety.

The maximum weight of tuber varied from 138 g (Lady Claire variety) to 395 g (Fabula variety). More than 300 g realized also the varieties Sprint (345 g) and Carrera (325 g).

The average yield in the two experimental years varied from 41.5 tons per hectare (Velox variety) to 60.3 tons per hectare (Fabula variety). The highest yield were determined by the weight of the tubers and not by the number of tubers, this been the case of Fabula variety. High yield (55.3 tons per hectare) was realized also at the Rosara variety, but in this case the yield was determined by the number of tubers per plant and not by the weight of tubers. In exchange, the Carrera variety realized a high yield (53.9 tons per hectare), this been determined both by number of tubers per plant and weight of tubers.

Varieties indicated for chipping (Lady Claire and Lady Rosetta) realized smaller yields than the other varieties. Varieties for chipping realized a high number of small tubers per plant.

The number of days from emergency to maturity varied between 75 (Velox, Rosara varieties) and 95 (Fabula variety). The highest yields were realized by the latest varieties (Fabula and Rosara), but Carrera variety which is an early variety realized also a high yield. Because the year 2007 was much warmer and dryer than 2006, the growth period was smaller with 5 days in average.
During the growth period, there were observed some specific characteristics that can be useful for the potato growers (table 1).

Table 1. Productive and some specific characteristics of the studied potato varieties (Average values for the years 2006 and 2007)

Nr. crt.	**Potato variety**	**Average no of tubers per plant**	**Average weight of tuber (g)**	**Maximum weight of tubers (g)**	**Yield (t/ha)**	**No of days from emergency to maturity (2006/2007)**	**Specific characteristics observed**
1.	Velox	10	78	245	41.5	80/75	Tubers are uniformly
2.	Fabula	6	188	395	60.3	95/90	Tubers do not have capacity to be stored and have to be commercialized very quickly
3.	Carrera	8	126	325	53.9	83/78	-
4.	Sprint	6	160	345	51.1	90/85	Tubers are not uniformly and about 40% of them are below the consume standard
5.	Rosara	13	80	245	55,3	80/75	-
6.	Lady Claire	14	61	138	42.1	85/80	This is sensible to the potato blight (*Phytophthora infestans*)
7.	Lady Rosetta	14	63	142	43,4	90/85	The tubers can be used for chips even before physiological maturity

Conclusions

4. The highest yield as average in the studied years (2006 and 2007) and under the specific conditions from South Romania were obtained at the Fabula variety (60.3 tons per hectare), this been determined especially by the weight of the tubers and not by the number of tubers.
5. High yield (55.3 tons per hectare) was realized also at the Rosara variety, but in this case the yield was determined by the number of tubers per plant and not by the weight of tubers.
6. Also, high yield (53.9 tons per hectare) was realized at the Carrera variety, this been determined both by number of tubers per plant and weight of tubers.
7. Varieties indicated for chipping (Lady Claire and Lady Rosetta) realized smallest yields compared with the other varieties.
8. Also, varieties for chipping realized a high number of small tubers per plant.

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PHYSICAL, PRODUCT AND SENSORY PROPERTIES OF POTATO TUBERS (*SOLANUM TUBEROSUM* L.) AS AFFECTED BY CULTIVATION SITE AND GENOTYPE

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Introduction

Specific pedo-climatic conditions of Italy allow potato cultivation in very different periods of the year. It is possible to distinguish an ordinary crop, carried out mainly in spring-summer cycle in the lowland (potato for consumption) and mountain ("seed" tuber) areas of central-northern Italy, and the off-seasonal crops, implemented in coastal areas of southern Italy and islands. The latter can be accomplished in two cycles: autumn-winter-spring and summer-autumn. The first allows the typical and well known early production (early potato) from March to June, while the second cycle allows obtaining winter production (from December to February) (Mauromicale and Ierna, 1999a; 1999b). The very marked environmental diversities, particularly regarding temperature, light and soil type, may affect the organographic features, physiological and productive plant process, as well as the tuber qualitative characteristics (Mauromicale *et al*., 2003).

Potato tubers from the autumn-winter-spring cycle, both for the particular environmental conditions in which the plants grow and above all since they are harvested and marked before their full "ripening" at a more or less advanced growth stage, have particular external and nutrient characteristics. These differ from tubers from ordinary cycle, which are harvested at complete "ripeness".

In this view, the aim of the present study was to evaluate the effects of cultivation site, which allows different crop cycles, and genotype on the physical, product and sensory characteristics of potato tubers.

Materials & methods

Plant material and experimental design

The field-experiments were conducted over two years (2006 and 2007) in Cetica (Tuscany), Ugento (Apulia) and Cassibile (Sicily), typical areas for potato cultivation in Italy. Three potato genotypes: "Rossa di Cetica", "Sieglinde" and "Arinda", typically cultivated in Tuscany, Apulia and Sicily, respectively, were selected.

In each year, planting was done manually in January (Apulia and Sicily) and in May (Tuscany), using whole disease-free seed-tubers and adopting a completely randomized block design with 4 replications. Each plot was 3.5 by 5.0 m with a planting density of 6.0 plant m^{-2}. Phosphorus (120 Kg ha^{-1}), potassium (120 Kg ha^{-1}) and nitrogen (50 Kg ha^{-1}) were applied in pre-sowing. 80 Kg ha^{-1} of nitrogen was supplied at tuber induction stage. Irrigation and pest management were in agreement with usual practice and uniform in the three locations.

Tubers were harvested at the end of the cycle (about 125 days after planting) when 70% of leaves were dry. A representative sample of marketable tubers (diameter 35-65 mm), belonging to the 3 genotypes and from the 3 localities under study, was selected and evaluated for the following analyses as fresh tubers or subjected to cooking procedures (boiling and frying).

Preparation of cooked samples

40 tubers for replication were peeled manually using a kitchen vegetable peeler, washed and drained.

30 tubers were boiled in a pressure cooker until soft (~ 15 min), cooled immediately in running cold water and kept at room temperature (20°C). For frying, a sample of ten pre-peeled tubers for replication was pushed through a vegetable slicer to obtain from the core region two sticks of uniform size (section 8 x 8 mm) trimmed to a length of 10 cm. After draining excess water, these sticks were fried in peanut oil at constant temperature of 180 °C for 5 min.

Physical measurements

Flesh colour evaluation was done in CIE L-a-b system, using a Minolta CR 300 colorimeter with illuminant D65, calibrated against a white-standard before each session.

Five fresh tubers for replication were cut longitudinally and immediately (zero time), in each half two readings, were made in the largest diameter area. In order to assess the possible phenomenon of enzymatic darkening, the same tubers, after being cut, were left at room temperature for 2 h and colour measurements were repeated. The differences in L, a and b values from zero-time readings were combined to obtain a total colour difference (ΔE) (Nourian *et al*., 2003). A larger ΔE means greater colour change from the reference material.

Cutting resistance evaluation on cooked potato samples was performed using a digital penetrometer (TR Turoni mod. 53205, Forlì, Italy) equipped with a specific shear probe tip. For each replication two measurements were made in the middle part of 10 sticks and in the core region of 5 boiled tubers cut in equal halves after cooling. The samples were placed perpendicular to the blade and the maximum force (cutting resistance), required for penetrating the samples to about 10 mm, was measured. Measurements were recorded in Newton cm^{-2} ($N\ cm^{-2}$).

Product evaluation

The appearance of the skin was estimated on the overall sample of marketable tubers through an index called "washability" (Lovatti *et al*., 1999), evaluated using a photographic scale prepared by CNIPT ITCF (1997) for the presence of external defects by mechanical, physiological and health origin, judged with a score range from 1 to 9 (very scarce – excellent).

Suitability to boiling was evaluated using the After Cooking Blackening (ACB) index. 5 tubers for replication, boiled as previously described and cut in halves, were evaluated for blackening degree using the following scoring system: 0= absence of the phenomenon; 1= slightly present; 2= widespread blackening.

For the assessment of culinary aptitude to frying, browning degree of 10 sticks, cooked as above described, was visually evaluated using the certified Munsell colorimetric paper. The values were adjusted from USDA to CISA category: 0= 000 extra white, no browning; 1 = 00 white or cream, no browning; 2 = 0 yellow, no browning; 3 = 1 light browning; 4 = 2 browning; 5 = 3 average-high browning; 6 = 4 complete browning.

Sensory evaluation

Sensory evaluation was performed with the method QDA (Quantitative Analysis Descriptor) (Hulrich *et al*., 2000) on 20 tubers of uniform size, boiled as previously described, with a panel of six judges trained for the following descriptors: consistency, dampness, granulation and typical taste. Panellists were asked to score the samples as the following: consistency (1= very tender, 2= quite tender, 3= moderate hard; 4= hard; 5= much hard), dampness (1= very dry; 2= quite dry, 3= quite humid; 4= wet; 5= very humid), granulation (1= very coarse; 2= fairly coarse; 3= fairly fine; 4= fine; 5= very fine) and typical taste (1- 5 = from absent to very pronounced).

Statistical analyses

All data were subjected to analysis of variance (ANOVA). The data reported are averages of two years, because the year effect was not significant.

Results

Generally, the parameters investigated (Tables 1-3) were significantly affected by the two factors under study, namely cultivation site and genotype, and by the "cultivation site x genotype" interaction. As regards the cultivation site, tubers from Cetica Plain showed better physical and product characteristics (Tables 1 and 2) than those from Cassibile and Ugento. Particularly, Cetica tubers reported the lowest total colour difference after cutting (ΔE = 4.3) and a good cutting resistance after boiling (2.6 $N\ cm^{-2}$), added to a good washability and an acceptable browning after frying (Table 2). Cassibile tubers, despite their highest cutting resistance of boiled tubers (3.5 $N\ cm^{-2}$), and Ugento ones showed the highest darkening after cutting (ΔE equal to 6.1 and 6.3, respectively) and also worst washability (5.5 and 5.8, respectively), compared to tubers harvested in Cetica.

Concerning genotype (Tables 1 and 2), "Arinda" achieved the highest washability (6.4) and the lowest total colour difference after cutting (ΔE=4.0). It also proved more suitable to boiling for high cutting resistance (3.0 $N\ cm^{-2}$) and low blackening of tubers after cooking (0.4). Instead, "Sieglinde" showed the worst physical characteristics, i.e. the highest ΔE (7.7) and the lowest cutting resistance after boiling (2.1 $N\ cm^{-2}$), even if it generally presented acceptable product properties, particularly due to its low blackening after boiling (0.5). "Rossa di Cetica" resulted more appropriate for processing into sticks, since it achieved on one hand the highest blackening after cooking (0.9), and on the other, high cutting resistance after frying (1.9 N cm^{-2}) and acceptable browning index (1.4). Sensory analysis (Table 3) confirmed the prevailing culinary aptitude of the 3 genotypes studied. In fact, "Arinda" and "Sieglinde" proved more suitable to boiling procedure, since they had delicate taste and showed rather tender, quite humid and fairly fine flesh after cooking. "Rossa di Cetica" appeared more appropriate for frying, because its flesh was judged quite dry and fairly coarse by panellists.

Considering the significance of "cultivation site x genotype" interaction, "Arinda" showed best results, in terms of physical and product characteristics, when tubers were harvested in Cetica and Cassibile. In fact, they reported lowest ΔE, highest cutting resistance after boiling and better washability compared to tubers from Ugento. This latter proved more suitable to boiling than tubers harvested in the other two localities investigated, thanks to better properties after cooking (delicate taste, quite humid and fairly fine flesh). "Rossa di Cetica" appeared more appropriate for frying when tubers were harvested in Cetica, since they resulted characterized by very dry and moderate hard flesh and stronger taste than boiled tubers from Ugento and Cassibile.

Conclusions

The results obtained in this experiment have mainly demonstrated how qualitative (physical, product and sensory) properties of fresh and cooked potatoes were significantly affected by cultivation site, genotype and interaction among these factors under study. Such results lead us to believe that it might be possible to define the qualitative profile of potato genotypes in relation to cultivation site, since the quality of tubers appeared to be affected by environmental conditions.

Ultimately, in consideration of the current trend in the agri-food field, further studies for identifying the qualitative parameters (chemical-nutritional, physical and sensorial) are still necessary to characterize local genotypes in order to promote potato production with the possible acquisition of IGP (Protected Geographical Indication) and DOP (Denomination of Protected Origin) labels and achieve a higher profile in the culinary destination of the product.

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Table 1. Physical measurements (colour and texture evaluation) of potato tubers as affected by cultivation site and genotype. NS = not significant.

Cultivation site	Total colour difference (ΔE)				Cutting resistance after boiling (N cm^{-2})				Cutting resistance after frying (N cm^{-2})			
	Ari	Ros di Cet	Siegl	**Mean**	Ari	Ros di Cet	Siegl	**Mean**	Ari	Ros di Cet	Siegl	**Mean**
Cetica	2.4	4.3	7.0	**4.3**	3.7	2.1	1.9	**2.6**	1.5	1.8	1.6	**1.7**
Ugento	6.3	3.7	9.0	**6.3**	1.5	2.1	2.0	**1.9**	1.6	2.0	1.7	**1.8**
Cassibile	3.4	7.8	7.0	**6.1**	3.8	4.3	2.5	**3.5**	1.5	1.8	1.5	**1.6**
Mean	**4.0**	**5.3**	**7.7**		**3.0**	**2.8**	**2.1**		**1.6**	**1.9**	**1.6**	
	LSD $P \leq 0.05$											
Cultivation site (C)	0.6				0.5				NS			
Genotype (G)	0.6				0.5				0.2			
Interaction (C x G)	1.0				0.9				NS			

Ari: "Arinda"; Ros di Cet: "Rossa di Cetica"; Siegl: "Sieglinde".

Table 2. Product evaluation of potato tubers as affected by cultivation site and genotype. NS = not significant.

Cultivation site	**Washability[1)]**				**After Cooking Blackening index[2)] (ACB)**				**Browning Index[3)] (BI)**			
	Ari	Ros di Cet	Siegl	**Mean**	Ari	Ros di Cet	Siegl	**Mean**	Ari	Ros di Cet	Siegl	**Mean**
Cetica	6.9	7.1	6.7	**6.9**	0.3	0.9	0.6	**0.6**	1.8	0.9	2.1	**1.6**
Ugento	5.9	5.1	6.5	**5.8**	0.2	1.4	0.5	**0.7**	1.4	2.2	1.8	**1.8**
Cassibile	6.6	5.6	4.5	**5.5**	0.7	0.5	0.5	**0.6**	0.3	1.1	0.9	**0.8**
Mean	**6.4**	**5.9**	**5.9**		**0.4**	**0.9**	**0.5**		**1.2**	**1.4**	**1.6**	
	LSD $P \leq 0.05$											
Cultivation site (C)	0.4				NS				0.3			
Genotype (G)	0.4				0.3				0.3			
Interaction (C x G)	0.6				0.6				0.5			

[1)] from 1 to 9 (very scarce – excellent); [2)] 0 = absence of the phenomenon; 1= slightly present; 2= widespread blackening;
[3)] 0= extra white; 1= white or cream; 2= yellow; 3= light browning; 4= browning; 5= average-high browning; 6= complete browning.

Table 3. Sensory evaluation of boiled potato tubers as affected by cultivation site and genotype. NS = not significant.

Cultivation site	**Consistency[1)]**				**Dampness[2)]**				**Granulation[3)]**				**Typical taste[4)]**			
	Ari	Ros di Cet	Siegl	**Mean**	Ari	Ros di Cet	Siegl	**Mean**	Ari	Ros di Cet	Siegl	**Mean**	Ari	Ros di Cet	Siegl	**Mean**
Cetica	2.3	3.0	2.6	**2.6**	2.4	1.2	2.4	**2.0**	3.5	2.2	3.1	**2.9**	2.6	1.7	2.1	**2.1**
Ugento	2.3	2.8	2.6	**2.6**	3.2	1.6	2.5	**2.4**	3.3	2.2	3.3	**2.9**	2.1	1.4	2.5	**2.0**
Cassibile	2.2	3.4	2.2	**2.6**	2.1	1.6	2.5	**2.1**	2.8	2.7	2.8	**2.8**	2.2	1.6	2.3	**2.0**
Mean	**2.3**	**3.1**	**2.5**		**2.6**	**1.5**	**2.5**		**3.2**	**2.4**	**3.0**		**2.3**	**1.6**	**2.3**	
	LSD $P \leq 0.05$															
Cultivation site (C)	NS				0.2				NS				NS			
Genotype (G)	0.2				0.2				0.2				0.1			
Interaction (C x G)	0.4				0.4				0.4				0.2			

[1)] 1= very tender; 2= quite tender; 3= moderate hard; 4= hard; 5= much hard; [2)] 1= very dry; 2= quite dry; 3 = quite humid; 4= wet; 5= very humid;
[3)] 1= very coarse; 2= fairly coarse; 3= fairly fine; 4= fine; 5= very fine; [4)] 1- 5 = from absent to very pronounced.

IMPROVING MARKET VALUE OF EARLY POTATO BY MEANS OF NUTRITIONAL TRAITS AND CULINARY SEGMENTATION (APULIA REGIONAL PROJECT "INNOVALO" – ITALY)

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Abstract.

The paper resumes general results of experimental trials and analysis conducted on early potato production in Southern Italy during the last three years, within the project "Innovalo" funded by the Apulia Region. The basic idea of the project is to add value to early potato tubers by means of relevant nutritional components and culinary segmentation, to meet market and consumer demands. General information about the importance of early potato production in Italy and in the Mediterranean countries are given. Materials and analytical methods are briefly described. At least about 30 new and old varieties suitable for early potato production have been compared in field trials, and tubers have been analyzed for: yield, tuber size distribution, dry matter, nitrate and vitamin C content, after cooking blackening, fry color, panel test for culinary segmentation. General results are described.

PHYSIOLOGY

THE INFLUENCE OF THERMAL SHOCK AND PRE-SPROUTING ON FORMATION OF YIELD STRUCTURE ELEMENTS IN EARLY POTATO VARIETIES

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Early potatoes proved to be profitable crops in Estonia. Varieties from the Estonian variety list are suitable for growing in our agro-climatic conditions despite the relatively short vegetation season. Pre-planting seed tuber treatment has enabled us to make early potatoes more profitable. Early varieties can provide a good yield, whereas quicker yield formation is characteristic of physiologically older plants. Pre-sprouting and thermal shock of seed tubers increase the physiological age of potato plants. The production of an early crop requires rapid plant emergence and foliage development, and tuber initiation at a low leaf area index. Physiologically older earlies are capable of developing sufficient leaf area for rapid assimilation. They also start accumulating nutrients into forming tubers.

Key words: leaf area index, physiological age, potato, tuber weight, tuber yield

Introduction

The 1990 growing area of potato in Estonia was 45,500 ha. Since then the growing area has suffered a remarkable decline to 30,900 ha in 2000 and to 10,200 ha in 2007 (Statistics Estonia, 2007). Concomittent to this decline has been the increase in imported potato which in recent years has reached 10–20 thousand tons per year.

The average consumption of potato, as foodstuff, per capita in developed countries, is usually 60–80 kg per year. Although the average consumption per capita of potato in Estonia has decreased by 28 kg, the annual figure remains in the 97–103 kg range (Statistics Estonia, 2003).

To supply the population with potatoes, it is important to cultivate varieties that provide a high quality yield as early as possible. To get an early potato harvest, growers cultivate special early-maturing varieties having a relatively short dormancy and developing sprouts already in February or March, while still being kept at a storage temperature of 5–10ºC. By early May the physiological maturity of seed tubers advances to a level where, after being planted into a soil that has a temperature of 7–10ºC, existing sprouts will start growing very fast and if the weather conditions are normal potatoes can be harvested 75–90 days after planting, i.e. in late July or early August. This is too late for the local consumers of earlies. Pre-sprouting and thermal shock add physiological age to potato tubers and therefore shorten the chronological period required to get a ripe yield of tubers (Allen *et al*., 1992).

We have focused our research on the effects of seed tubers with different age on the different parts of a potato plant. If the seed tubers are kept for certain time at higher temperatures before planting, physiologically older tubers are obtained. It is important while growing early or late potato varieties because maximum weight of the haulms and the leaf area index (LAI) are attained faster and it is possible to harvest also the economically optimal tuber yield much earlier.

Material and methods

An experiment was carried out by the Department of Field Crop Husbandry of the Estonian University of Life Sciences, to investigate the possibilities of different pre-planting seed tuber treatment methods. The early varieties 'Maret' (bred at Jõgeva Plant Breeding Institute) and 'Vineta' (bred at Europlant (Kartoffelzucht Böhm)) were used in the experiment. Different options of growing potato were analysed, using various pre-planting treatments on seed tubers, as follows:

1. Untreated variant (T_O) – thermal treatment was not conducted, the seed tubers were stored in

darkness at a temperature of 4–6°C;

2. Thermal shock (T_S) – the seed tubers were stored in darkness at a temperature of 4–6°C, then a week before planting the tubers were kept in a brightly lit room for 2 days at 30°C and a further in a dimly lit room 5 days at 12–15°C;

3. Pre-sprouting (P_S) – the seed tubers were stored in darkness at a temperature of 4–6°C, then 35–38 days before planting were kept in a sufficiently humid (85–90%) and in a dimly lit room at 12–15°C.

The soil of the experimental field was *Stagnic Luvisol* by World Reference Base for Soil Resources 1998 classification with a texture of sandy loam and a humus layer of 20–30 cm (Reintam & Köster, 2006). Agrotechnics were typical of potato experiments.

The dynamics of the tuber yield, the weight of the haulms, leaf area, the number of tubers per plant and the average weight of a tuber was determined with the interval of 3–5 days and each sample consisted of 4 plants from the test plot.

The results were statistically improved using the regression analysis method with the following quadratic equation: $y = a + bx + cx^2$, wherein: y – argument function, the index that is calculated on the basis of the equation: tuber yield, the weight of the haulms, LAI, the number of tubers per plant and the average weight of a tuber, a – constant term of the equation, b and c – regression coefficients, x – argument; number of days after planting.

In this paper all the experimental data are presented on the average of two varieties.

Results and discussion

Earlier studies at the Department of Plant Production indicated that P_S and T_S increased the physiological age of seed tubers and initiated earlier emergence (Jeremejev *et al*., 1998; Jeremejev *et al*., 1999; Lõhmus *et al*., 1999). Plants from seed tubers of late cultivars treated with T_S emerged 1–4 days earlier than T_O variant (Jeremejev *et al*., 1998; Jeremejev *et al*., 1999; Eremeev *et al*., 2001). In present study, the pre-planting treatment of early varieties seed tubers ensured earlier field sprouting in the T_S variant by 2, and in the P_S variant by 7, days earlier than in untreated variant.

With thermal treatment of seed tubers we increase the physiological age of these tubers and it has strong influence on yield structure elements of potato plant. In plants developed from physiologically older tubers the LAI (2.08 units) is formed already on the 45 DAP and it is larger, compared to T_O variant (0.75 units LSD_{05} 0.5) and T_S variant (0.73 units LSD_{05} 0.63). In P_S variant, the statistically significant maximum LAI is formed after 80 day after planting (DAP) (5.51 units) that is bigger than T_O and T_S variant (by 1.06 and 0.70 units, respectively).

Table 1. The effect of the potato variety on the leaf area index

Days after planting	Untreated (T_O)	Thermal shock (T_S)	Pre-sprouting (P_S)
45	1.33a*	1.35a	2.08b
50	2.13a	2.28a	3.05b
60	3.39a	3.70a	4.51b
70	4.16a	4.54a	5.33b
80	4.45a	4.80a	5.51b
90	4.26a	4.48ab	5.04b
100	3.59a	3.58a	3.94a
110	2.44a	2.10a	2.19a
115	1.69a	1.15b	1.08b
n[1]	44	44	42
SE[2]	0.36	0.59	0.57
CL_{05}[3]	0.19	0.32	0.31

* = Means followed by the some letter in the some row are not significantly different ($p < 0.05$)
[1]*n* = number of samples
[2]SE = Standard error
[3]CL_{05} = Confidence limits at $p = 0.05$

Pre-sprouting had significant influence on the number of tubers per plant until 80 DAP, compared to

T_S and T_O variant (Table 2). By that time there were 16.8 tubers in P_S variant, 14.0 tubers in T_S variant and 14.3 tubers in T_O variant (differences statistically significant). Compared to T_O variant, the P_S variant had strong influence on the average weight of the tuber. There were significantly bigger tubers in P_S variant until 90 DAP than in T_O variant, 58.8 and 52.8 g respectively (Table 3).

Table 2. The effect of the potato variety on the number of tubers per plant

Days after planting	Untreated (T_O)	Thermal shock (T_S)	Pre-sprouting (P_S)
60	10.0a*	10.5a	14.6b
70	12.8a	12.6a	16.1b
80	14.3a	14.0a	16.8b
90	14.7a	14.6a	16.6a
100	13.8a	14.4a	15.5a
110	11.7a	13.3a	13.6a
120	8.4a	11.5b	10.8b
n^1	36	38	38
SE^2	1.9	1.3	2.5
$CL_{05}{}^3$	1.1	0.7	1.4

* = Means followed by the some letter in the some row are not significantly different ($p < 0.05$)
[1]*n* = number of samples
[2]SE = Standard error
[3]CL_{05} = Confidence limits at $p = 0.05$

Table 3. The effect of the potato variety on the tuber weight

Days after planting	Untreated (T_O)	Thermal shock (T_S)	Pre-sprouting (P_S)
60	10.7a*	14.4a	19.2b
70	25.2a	30.3b	34.0b
80	39.2a	44.4b	47.2b
90	52.8a	56.7ab	58.8b
100	65.9a	67.2a	68.8a
110	78.7a	76.0a	77.1a
120	90.9a	82.9b	83.8b
n^1	36	38	38
SE^2	3.7	3.9	5.0
$CL_{05}{}^3$	2.0	2.1	2.7

* = Means followed by the some letter in the some row are not significantly different ($p < 0.05$)
[1]*n* = number of samples
[2]SE = Standard error
[3]CL_{05} = Confidence limits at $p = 0.05$

Table 4. The effect of the potato variety on the tuber yield

Days after planting	Untreated (T_O)	Thermal shock (T_S)	Pre-sprouting (P_S)
60	6.9a*	11.2b	16.6c
70	20.6a	24.4b	30.3c
80	31.9a	35.5b	41.3c
90	40.7a	44.7b	49.8c
100	47.0a	51.8b	55.5c
110	50.9a	56.9b	58.7b
120	52.2a	60.1b	59.2b
n^1	36	38	38
SE^2	1.9	3.3	2.6
$CL_{05}{}^3$	1.0	1.8	1.4

* = Means followed by the some letter in the some row are not significantly different ($p < 0.05$)
[1]*n* = number of samples
[2]SE = Standard error
[3]CL_{05} = Confidence limits at $p = 0.05$

Thermally treated variants yielded significantly better than T_O variant. The yield of P_S variant was statistically higher until 100 DAP (LSD_{05} 3.2) than in T_S variant (Table 4).

Conclusions

It is beneficial to use P_S seed tubers when growing early potato varieties. The plant from physiologically older seed tubers develops through different growth stages faster than younger tubers. P_S variant enables the tubers to form earlier and there are more and heavier tubers. Also the leaf area forms earlier and it is bigger which in the end results in higher yield than T_O and T_S variant.

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INFLUENCE OF VARIOUS FERITLIZATION LEVELS TO INCREASE CONTENT OF ANTIOXIDANTS IN POTATO TUBERS

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The objective of several years of field experiments was to explore the changes in the total polyphenol and ascorbic acid content, as important antioxidants, in potato tubers at various levels of N, P, K and Mg nutrition. The polyphenol and ascorbic acid content was affected particularly by the weather in the experimental year; the fertilization variants had no significant effect on the polyphenol content. A negative effect on ascorbic acid content in tubers was observed in the case of an increased intensity of N fertilization (at 180 kg N/ha ascorbic acid decrease was lower by 6,1 % compared to doses 100 kg N/ha). On the contrary, a favourable effect was determined at increased levels of potassium and magnesium fertilization (at 166 kg K/ha and 60 kg Mg/ha ascorbic acid increase was by 6,2 % higher compared to levels of 108 kg K/ha and 30 kg Mg/ha).

Keywords: **potatoes, antioxidants, ascorbic acid, polyphenols, nutritional quality**

Introduction

The potato tuber contains many substances important with regard to the rational human nutrition. These substances form the nutritive value of potatoes and are often of the importance as compounds contributing to final taste and flavour of a ready product (9). Concrete quality of potato tubers and potato products is affected by large amount of substances. First, they are substances with positive influence, which can contribute to increase of the nutritional quality (2). Their presence together with vitamins and provitamins is considered as a key point in regard of chronic disease prevention such as cancer, cardiovascular diseases or diabetes at present. Many of these substances even indicate higher antioxidant effects than vitamins C and E. These food antioxidants with these vitamins form bioactive mechanisms, which remove free radicals and decrease oxidative stress (5). From substances with a negative effect on quality we could mention especially extraneous substances e.g. pesticide residues, polychlorinated biphenyls (PCB), nitrates and trace amounts of risk elements, particularly heavy metals (2).

Potato tubers represent the most important sources of antioxidants in the human nutrition (1). For example, on average, about 64 mg of polyphenols per capita contribute to providing of daily intake in the US and potato tubers are ranked on the second place after tomatoes (11). Natural antioxidants present in potato tubers and other food staff give rise to large interest at present due to their potential nutritional and therapeutic effects. Polyphenols (1226-4405 $mg.kg^{-1}$) and L-ascorbic acid (170-990 $mg.kg^{-1}$) are the most contained substances in potato tubers. Other contained antioxidants are carotenoids, α-tocopherol and smaller amount of selenium and α-lipolic acid (7).

Content of individual antioxidants is presupposed to be positively affected. Stress factors such as mechanical tuber damage, pathogen infection and acting of light on tubers (4) have the highest effect on polyphenol content. From factors, which could be used, we could positively affect polyphenol content especially by selection of suitable varieties (13), selection of locality (8), storage (6), suitable N and K ratio of fertilization and reduction of polyphenol losses by an appropriate culinary treatment (3). Ascorbic acid content could be positively affected by the selection of suitable varieties; between individual varieties there could be found large differences in regard of ascorbic acid decline during storage (12). Potassium fertilization in the form of K_2SO_4, phosphorus, magnesium fertilization has a favourable effect on ascorbic acid content, while KCl and nitrogen fertilization has a negative effect.

Material and methods

Field trials were established in the experimental locality Valečov. Two ware potato varieties were used for planting differing in maturity group – early Karin and medium-early Ditta. An effect of various nutrition doses on total phenol (2004-2006) and ascorbic acid content (2004-2006) was studied. Following fertilization variants (Tab. 1) were used for the trial: N/P/K/Mg: 0/0/0/0, 100/44/108/30, 100/44/166/60, 180/44/108/30 kg net nutrients.ha^{-1}. Tubers for all chemical analyses were sampled in the beginning of crop physiological maturity, i.e. in the period of maximum harvest of given varieties for the Czech market. In case of total polyphenols (CP) samples were frozen after the harvest and then lyophilised. After the lyophilisation spectrophotometric analyses were initiated with phenolic Folin-Ciocalteu reagent on Helios spectrophotometer. For ascorbic acid (AA) determination samples were placed into the cooling box and analysed in the fresh state by a polarographic approach – polarographically on micropolarograph Eko-Tribo Polarosensor.

Results and discussion

Results of total polyphenol content in potato tubers in dependence on a fertilization variant are given in Tab. 2. A statistical highly significant effect of year was found, when higher total polyphenol content was 4,78 mg.g^{-1} lyophilisate in 2005 compared to 3,11 mg.g^{-1} lyophilisate in 2004. Various fertilization variants had no statistical significant effect on total polyphenol content. On contrary, Radi et al. (14) refer to an increase in phenolic substance content in fruits after increase of potassium dose from 60 to 120 kg.ha^{-1} and decrease of nitrogen dose from 150 to 80 kg N.ha^{-1}.

Results of ascorbic acid content are given in Tab. 3. This content was statistically significantly affected by year and also fertilization variant. A positive effect on ascorbic acid content was found with increasing of K dose to 166 kg.ha^{-1} with simultaneous increasing of Mg dose to 60 kg.ha^{-1}. In 2004 only tendency was found; however, in 2005 and also in average of both years the result was significant. A favourable effect of potassium fertilization on ascorbic acid content is consistent with authors' conclusions: Nowacki et al. (18) and Mondy and Munshi (19). Statistical significant higher ascorbic acid content was recorded for the variant 2 compared to the variant 4. It could be concluded that higher nitrogen dose (var. 4) negatively affected ascorbic acid content in tubers. This is also confirmed by Lee Seung and Kader Adel (15), who refer that higher nitrogen doses reduce vitamin C content in many fruits and vegetables. On contrary, Smatanová (16) describes that nitrogen doses were not expressed in vitamin C content changes in tissues of spinach. Also Premuzic et al. (17) refer that various doses and forms of nitrogen did not significantly affected vitamin C content in lettuce.

Summary and conclusion

The obtained results show that polyphenol and ascorbic acid content in potato tubers was affected especially by the experimental year. Since in case of polyphenols fertilization variants did not have any significant effect on their content, the variant with nitrogen dose caused a decrease in ascorbic acid content. Increasing of nutrition important substance content, incl. natural antioxidants in potato tubers belongs to possibilities how to achieve their optimal daily intake in human diet and preventively act against so-called civilization diseases.

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Table 1: Fertilization variants with applied nutrient doses (kg.ha^{-1})

Variant	N	P	K	Mg
1	0	0	0	0
2	100	44	108	30
3	100	44	166	60
4	180	44	108	30

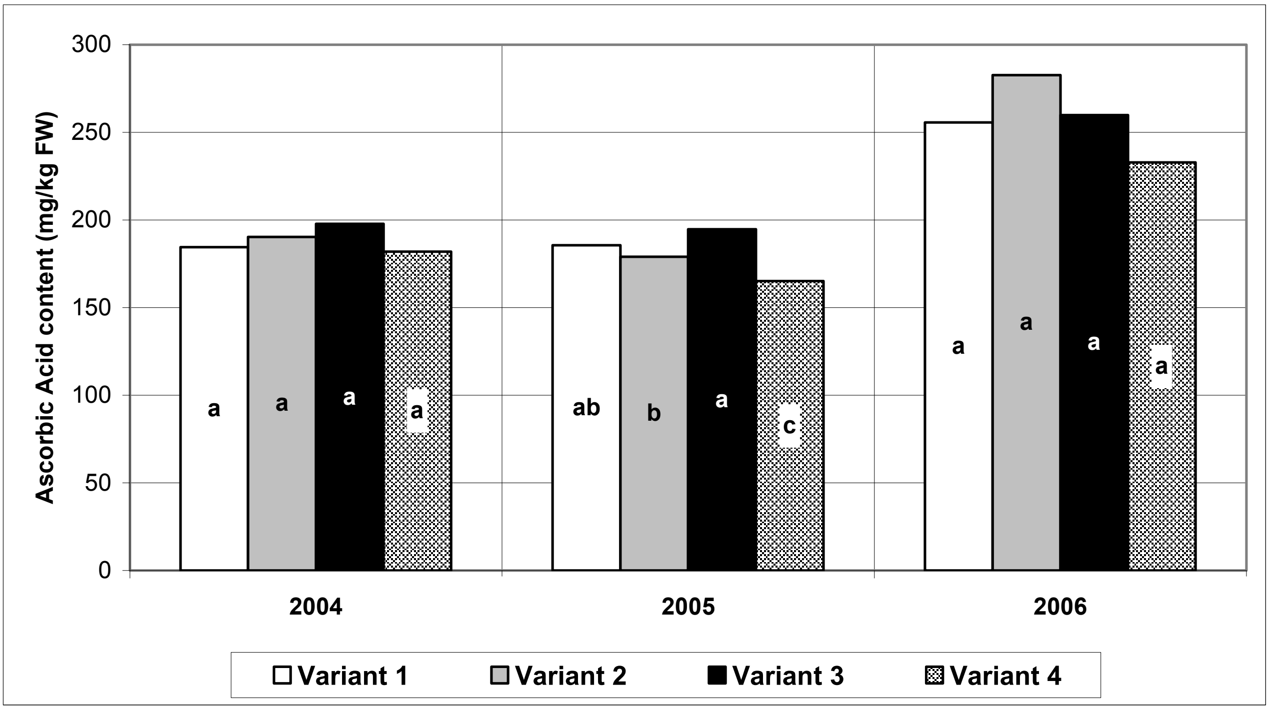
Table 2: Total polyphenol content in potato tubers (mg. g^{-1} lyophilisate)

	Variant						Year				
	1	2	3	4	$Dt_{0,05}$	$Dt_{0,01}$	2004	2005	2006	$Dt_{0,05}$	$Dt_{0,01}$
Polyphenol content	4,013	3,957	3,853	3,963	0,345	0,431	3,106	4,785	3,950	0,266	0,388

Table 3: Ascorbic acid content in potato tubers (mg.kg^{-1} fresh matter)

	Variant						Year				
	1	2	3	4	$Dt_{0,05}$	$Dt_{0,01}$	2004	2005	2006	$Dt_{0,05}$	$Dt_{0,01}$
Ascorbic acid content	231,01	252,04	239,25	215,94	28,59	35,77	188,68	258,00	257,75	22,07	32,20

Figure 1 Effect of the level of mineral fertilization (fertilization variants) on the content of ascorbic acid (mg.kg^{-1} fresh weight) in Valečov locality



Vertical lines represent SD (4 replicates), means with same letter are not significantly different ($P \geq 0.05$)

Var. 1 without fertilization with mineral fertilizers
Var. 2 100 kg N/ha, 44 kg P/ha, 108 kg K/ha, 30 kg Mg/ha
Var. 3 100 kg N/ha, 44 kg P/ha, 166 kg K/ha, 60 kg Mg/ha
Var. 4 180 kg N/ha, 44 kg P/ha, 108 kg K/ha, 30 kg Mg/ha

EFFECT OF LOCALITY AND VARIETY ON TOTAL STARCH CONTENT AND YIELD AND AMYLOSE CONTENT OF POTATO VARIETIES FOR INDUSTRIAL PROCESSING

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The aim of exact field trials was to verify the ability of potato varieties (22) to provide relatively stable tuber starch content with regard to length of growing period and amylose content in potato starch in selected localities (5) in the Czech Republic. Two-year results show that the effect of all studied factors on tuber starch content exceeded significance limit. Localities (F = 42,75**) and varieties (F = 28,02**) were the most expressed factors. Year was relatively less expressed (F = 8,29**). For localities, starch content varied on average between 19,18 % (Vysoké nad Jizerou) and 21,40 % (Valečov) and for years between 20,15 % (2006) and 20,48 % (2005). For varieties, range of starch content was between 18,21 % (medium-late Saturna for chip and starch production) and 22,70 % (medium-late Amylex for starch production). Amylose content was significantly affected by year (F = 16,50**), variety (F = 10,14**) and also locality (9,97**). Averaged over localities amylose content ranged between 20,68 % (Horažďovice) and 22,07 % (Vysoké nad Jizerou), i.e. the highest content was recorded in the locality that revealed on average the lowest starch content. However, this finding was not confirmed averaged over years (21,01 % in 2005 and 21,62 % in 2006) and also in individual varieties. In case of varieties amylose content had relatively wide range, between 18,61 % (very late Amado) and 22,84 % (early Orbit) with a certain tendency of higher content in varieties with shorter growing period. The results of the study show that unambiguous positive relation of growing period duration and tuber starch content was not confirmed. Simultaneously, critical effect of relatively high tuber yield on starch content was apparent. A positive interaction between starch content and tuber yield was only recorded for the variety Westamyl and this was reflected in starch yield. Therefore, starch yield was economically interesting for growers and also processors.
Keywords: potatoes; variety; locality; year; starch content; starch yield; amylose content

Introduction

Potato starch production from 1 hectare is particularly associated with the amount of tuber yield and tuber starch content. Amount of yield is affected by yield-forming components and grower's ability to utilize them for yield formation in individual varieties (VOKÁL et al. 2004). Amount of tuber yield has also an important effect on starch yield. In general, it is supposed that tuber yield acts more important than tuber starch content with regard to starch yield. The grower can act on amount of tuber yield to a greater extent than on starch content. Variety significantly influences starch content and its characteristics (MÍČA 1988, EZEKIEL et al. 2007) and therefore breeding activity (DOMKÁŘOVÁ et al. 2007) is directed to development of varieties with high starch content. The results are varieties with combination of high starch content and ability to reach relatively high tuber yielding level. This presupposition is especially realized in case of varieties with longer growing period (medium-late, late, very late), although varieties with shorter growing period reaching required level of starch content (over 20 %) are also available and allow processing industry to distribute processing into longer period with even offer of raw staff. Varieties with shorter growing period give lower tuber yield and also lower starch yield. The length of growing period belongs to important factors, then very late and/or late varieties must be harvested in right time, i.e. in the period, when weather is favourable for mechanized harvest. Real danger exists that growing (weather) conditions do not allow creating of presuppositions for utilization of genetic potential for formation of high tuber yield and also high tuber starch content and vegetation has to be ended much earlier before physiological maturity of the crop is reached.

A potato tuber contains starch in two different forms, i.e. amylose and amylopectin (1:4 to 1:5). For industrial use (for example basic compound for films, adhesives and packages) and also for certain starch additives amylopectin-starch is especially attractive. Separation of both starch components during industrial processing is very expensive and results in high amount of waste waters. Thus, people turn back to plants with high amylose or amylopectin content according to respective need.

Materials and methods

In exact field trials in five localities during 2005-2006 (Domanínek, Horažďovice, Lukavec, Valečov, Vysoké) the effect of 22 varieties (Albatros, Amado, Amylex, Amylon, Apolena, Ikar, Krumlov, Kuras, Nomade, Oktan, Orbit, Ornella, Rebel, Roberta, Saurna, Sibu, Sírius, Sonate, Tábor, Tomensa, Vladan, Westamyl) was studied on total tuber starch yield and amylose (amylopectin) content.

The aim of the study was to determine the effect of year, variety and locality on mentioned indices and to evaluate significance of acting of individual factors based on a statistical assessment.

Field trials were carried out with uniform cultural practices, protection and fertilization. Plots (1,5 m x 9,3 m = 2 rows with 32 hills each) were manually planted at distance 0,75 x 0,29 m with certified chitted seed potatoes of 35 – 45 mm. Three replications were performed.

Total starch content from average sample of harvested tubers was determined in department of VÚB in Valečov using Hošpes-Petzold weighs, amylose content was measured in laboratory of analytical chemistry of VÚB using modified Con A method developed by YUN and MATHESON (1990).

Results and discussion

The effect of variety, year and locality on total starch content, starch yield and amylose content in potato starch was highly statistical significant in all cases (Tab. 1).

Tab. 1 Analysis of variance for evaluation of effect of individual factors

Index	Starch content			Starch yield			Amylose content		
	variety	year	locality	variety	year	locality	variety	year	locality
F – tesf of significance	28,02	8,29	42,75	7,65	268,47	73,1	10,14	16,56	9,97
	**	**	**	**	**	**	**	**	**
Tukey test									
- 0,05	1,391	0,227	0,505	1,847	0,301	0,474	1,809	0,295	0,657
- 0,01	1,578	0,302	0,61	2,094	0,392	0,572	2,052	0,392	0,793

Evaluating the effect of **year** higher starch content (20,48 %) was recorded on average of the whole set in 2005. It is an unambiguous tendency, since this fact was only confirmed at the workplace in Vysoké nad Jizerou (further Vysoké) and to certain extent also in Horažďovice. It is concluded that a difference in favour of 2005 was especially associated with a significant difference in Vysoké, in that this tendency was also confirmed in all varieties. An explanation could be found in various rainfall distributions in the end of growing period. Rainfalls in August and September amounted 155,2 and 260,5 mm in 2005 and 2006, resp. An excess of water in this period probably delayed maturity of crop and also starch accumulation in tubers. In case of starch yield, position of the year 2005 was unambiguous on average of the set and mean starch yield (12,34 $t.ha^{-1}$) highly significantly exceeded level of the year 2006 (by 25,2 %). This result also confirmed (VOKÁL et al. 2004) the decisive importance of tuber yield for total starch yield. Amylose content highly significantly exceeded level of the year 2005 (21,01 %) on average of the whole set in 2006 (21,62 %). It is interesting that this statement was on average not true only for Vysoké (level of the year 2005, i.e. year with higher starch content was on average significantly higher).

Considering **localities** highly significant increase of starch content was on average determined in Valečov (21,40 %), namely comparing with all other localities, the most significant with regard to Vysoké (19,18 %). Even this level did not balance lower tuber yield, determined in Valečov and this workplace found on average the lowest level of starch yield (9,46 $t.ha^{-1}$). Decrease in Valečov (and also in Vysoké) was highly significant to starch yields found in Domanínek, Horažďovice and Lukavec. The highest amylose content was on average found in Vysoké (22,07 %). The lowest one was recorded for Horažďovice (highly significant decrease to 20,68 %) and for Valečov (also highly significant to 21,00 %).

On average, starch content over 22 % was determined in the **varieties** Amylex (22,70 %), Westamyl (22,47 %), Roberta (22,36 %) and Amylon (22,17 %). Starch content over 21 % was found in the varieties Ikar (21,96 %). Albatros (21,56 %) and Rebel (21,52 %). It is interesting that varieties with shorter growing period (Rebel – early, Albatros and Roberta – medium-early) also belong to the best ranked varieties. Variety Saturna (18,21 %), Sonate (18,26 %), Nomade (18,48 %) and Oktan (18,67 %) are below limit of 19 % and they are also varieties with wide range of growing period duration.

Considering varieties, starch yield again confirmed the decisive effect of tuber yield on this index. Starch yield over 12 $t.ha^{-1}$ was on average recorded for the variety Kuras (13,38 $t.ha^{-1}$), Westamyl (12,48 $t.ha^{-1}$), Amado (12,30 $t.ha^{-1}$), Vladan (12,12 $t.ha^{-1}$), Sibu (12,09 $t.ha^{-1}$) and Krumlov (12,02 $t.ha^{-1}$). From these varieties only Westamyl reached mentioned level with higher tuber starch content (22,47 %). Starch content of other varieties ranged between 19,23 % (Krumlov) and 20,05 % (Sibu).

Relatively lower level of starch yield was determined in medium-late variety Apolena and Amylex and early variety Tomensa.

Amylose content was relatively equal on average of the set of evaluated varieties, on contrary to finding of EZEKIEL et al. (2007), PHADNIS et al. (1991), KIM et al. (1995) and others. It varied between 18,61 % (very late Amado) and 22,84 % (early Orbit). The highest values were significantly lower than findings of GANGA et al. (1999), PHADNIS et al. (1991) and KIM et al. (1995). In their trials there were series of varieties with content around 30 % and/or varieties with content over 35 %. Our finding nears to the data of EZEKIEL et al. (2007) and LESZCZYNSKI (1989) showing 16 – 24 %. In their work they also refer to the effect of genotype, growing conditions and analytical methods on level of determined values. Mean amylose content (21,32 %) determined in our trials is comparable to the value (21,0 %) found by SWINKELS (1985) and also EZEKIEL et al. (2007), who found amylose content on average on the level of 20,5 %. Only two varieties (Amado and Sonate) revealed amylose content below 20 % in our trials, i. e. amylose : amylopectin ratio more than limit 1 : 4. In addition to variety Orbit also variety Oktan (22,72 %), Apolena (22,69 %), Sibu (22,60 %), Vladan (22,30 %), Krumlov (22,19 %) and Ornella (22,16 %) belonged to the varieties with lower amylopectin portion (i.e. less than limit 1 : 3,5). Variety Westamyl with relatively extraordinary position with regard to starch content and yield revealed on average amylose content of 21,07 %, i.e. amylose : amylopectin ratio 1 : 3,75.

Conclusion

The results of the study show that unambiguous positive relation of growing period duration and tuber starch content was not confirmed. Simultaneously, critical effect of relatively high tuber yield on starch content was apparent. A positive interaction between starch content and tuber yield was only recorded for the variety Westamyl and this was reflected in starch yield. Therefore, starch yield was economically interesting for growers and also processors.

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ECOPHYSIOLOGICAL RESPONSE OF POTATO GROWN IN MEDITERRANEAN ENVIRONMENT UNDER DIFFERENT NITROGEN RATE

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INTRODUCTION

Nitrogen fertilization with mineral forms is the most important nutrient input in agriculture (Navarro Pedreño et al., 1996). It affects leaf area index (LAI) of potatoes which influence the seasonal pattern of light interception and crop production (Vos and Van der Putten 1998). Crop yield is also dependent on radiation use efficiency (RUE); increased nitrogen supply stimulates photosynthetic capacity of leaves, through increases in stromal and thylakoid proteins in leaves (Evans and Terashima 1988; Evans 1989). The ability of plants to assimilate CO_2 is in part a function of the rate of electron transfer occurring through photosystems (Strong et al., 2000), thus the chlorophyll fluorescence of a leaf can be used as an indicator of photosynthetic activity (Schreiber et al., 1995).

In south Italy, as in other southern coastal areas of the Mediterranean basin, the potato crop is realised during the winter-spring cycle (from November-December to May-June) to obtain off-season production. In these environmental conditions information on the chlorophyll fluorescence response to nitrogen supply would be useful to improve knowledge on mechanisms that control tuber yield, as well as allow a possible saving of nitrogen which is an expensive and serious pollution problem as a consequence of its groundwater leaching.

The objectives of this research were to study the chlorophyll fluorescence response of early potato crop to nitrogen rate and genotype and to analyse the possible relation between chlorophyll parameters and yield, in order to explore the possibility of applying this physiological approach to agronomic studies in the field.

MATERIALS AND METHODS

Field experiment was conducted during 2004 along the coastal plain area south of Siracusa (37°03' N, 15°18' E, 10 m asl), which is a typical area for early potato cultivation in south Italy. The climate in this area is semi-arid-Mediterranean, with mild winters and often rainless springs. The soil type is calcixerollic xerochrepts (USDA, Soil Taxonomy), moderately deep, with a loam-clay texture.
The experiment was arranged in a randomised split-plot design with four replications including 5 nitrogen rates (0, 100, 200, 300 and 400 kg ha^{-1}) as main plots and 3 genotypes (Arinda, Rubino and Ninfa) of potato (*Solanum tuberosum* L.) as sub-plots. Sub-plots size was 4.2 by 4.2 m, with 84 plants. Nitrogen was supplied ¼ pre-planting as ammonium sulphate and ¾ two weeks after emergence as ammonium nitrate.

Arinda is the most widely cultivated genotype in the Mediterranean region; it is a ware potato, of early maturity with long, regular and very large tubers; plants produce few, but erect and vigorous, stems. Rubino (ex ISCI 4052) is a new Italian variety, of early maturity with short, oval and regular tubers; stems are of medium size. 'Ninfa' (ex ISCI B 26) is also a new Italian variety, of medium to late maturity, with oblong, regular and very large tubers; stems are fairly tall and erect (Ranalli et al., 2005).

Planting was done manually on 15 January using whole disease-free tubers. Phosphorus (100 Kg ha^{-1}), potassium (150 Kg ha^{-1}) and chlorpyrifos (30 kg ha^{-1}) were applied before planting. All plants emerged. Drip irrigation was carried out when the accumulated daily evaporation reached 30 mm and supplying 100% of maximum evapotranspiration. The usual crop management was used: pest emergence with linuron and pest control when needed.

Chlorophyll measurements

Chlorophyll fluorescence parameters (F_0, F_m, F_v, F_v/F_m, T_{max}) were recorded in the field with a portable fluorescence induction monitor (Fi_m 1500, Alma Group Company, Pindar Road, Hoddesdon, Herts, England). Measurements were made on the terminal of the youngest fully expanded leaf after a 20 min dark adaptation period between 11 a.m. and 1 p.m. (local solar time).

Relative chlorophyll content was measured in the field using a portable chlorophyll meter (SPAD 502, Minolta Camera, Osaka, Japan). Triplicate readings were taken at each leaf of the four plants previously marked for chlorophyll fluorescence measurements. Both measurements were taken on four sunny days during tuber growth.

Yield measurement

The harvest area consisted of 20 plants from the center of each sub-plot. Plants were harvested by hand on 19 May, when about 70% of haulm was dry and weight of tubers was measured.

Statistical analysis

All data were submitted to Bartlett test for the homogeneity of variance and then were analysed using analysis of variance (ANOVA) as a factorial combination of nitrogen rate x genotype x measurement date. Means were compared by *LSD* test, provided the *F* test was significant. Polynomial effects up to second degree were made where appropriate to define the response of trend (linear or quadratic) between nitrogen supply and chlorophyll fluorescence parameters and chlorophyll content.

RESULTS

Results of the averages of measurements made on the four days are reported. The effects of main factors were considered.

Chlorophyll content

The chlorophyll content showed significant differences in relation to nitrogen rate and genotype; it increased significantly and linearly with increasing nitrogen rates until the maximum dose studied. It increased, however, by about 30% passing from 0 to 100 kg ha^{-1}, but only by 3% passing from 100 to 400 kg ha^{-1}. 'Arinda' exhibited a higher chlorophyll content (on average, 46.3 SPAD) compared to those of 'Ninfa' (44.0 SPAD), and 'Rubino' (43.5 SPAD) (Table 1).

Chlorophyll fluorescence parameters

All chlorophyll fluorescence parameters were influenced only by nitrogen rate, excepting T_{max} which was affected by 2 factors

Initial fluorescence (F_0), which represents the basal emission of chlorophyll fluorescence when redox components of photosystems are fully oxidised, was equal to 661 relative units in the control and decreased significantly with 100 kg ha^{-1} of nitrogen, reaching an intermediate level at the highest doses of nitrogen kg ha^{-1} (Table 1). Maximum fluorescence (F_m) which is obtained at the fully saturated light intensity for the plant when the electron acceptor QA is fully reduced, was in the control equal to 1874 relative units, and increased on average by 18% with the input of nitrogen without differences between the doses. Variable fluorescence (F_v) which was obtained by subtracting F_0 from the F_m value, equal to 1235 relative units in the control significantly and linearly ($P<0.001$) increased with the input of nitrogen until the maximum dose (400 kg ha^{-1}). F_v/F_m , which has been shown to be proportional to the quantum yield of PSII photochemistry and exhibits a high degree of correlation with the quantum yield of net photosynthesis, was lowest in the control (0.636) but increased significantly in nitrogen fertilized plots. T_{max}, which is the time at which the maximal fluorescence occurs, increased ($P < 0.05$) with 100 kg ha^{-1} of N to then show intermediate values at higher doses. Furthermore, T_{max} was smaller in Rubino compared with the other genotypes (Table 1).

Relationship between chlorophyll fluorescence parameters and yield

Fresh tuber yield was positively and significantly associated with F_v/F_m (r= 0.661**) and with chlorophyll content (r=0.859***) (Figure 1).

DISCUSSION AND CONCLUSIONS

Under the particular conditions in which the experiment was conducted, chlorophyll content and all chlorophyll fluorescence parameters were significantly affected by nitrogen rate, proving to be valid in detecting differences in potato leaf photosynthesis.

Table 1 – Chlorophyll content and chlorophyll fluorescence parameters of field grown potato as affected by nitrogen rate and genotype. Different letters within each factor (nitrogen rate or genotype) and parameter, indicate significant differences for P<0.05.

Treatment	Chl content (SPAD)	F_0 (rel units)	F_m (rel units)	F_v (rel units)	F_v/F_m	T_{max} (ms)
Nitrogen rate (kg ha^{-1})						
0	35.6 c	661 a	1874 b	1235 c	0.636 b	224 d
100	46.1 b	615 b	2198 a	1592 ab	0.716 a	307 a
200	47.1 ab	653 a	2202 a	1570 b	0.699 a	278 bc
300	46.5 ab	658 a	2218 a	1562 b	0.700 a	273 c
400	47.6 a	639 ab	2259 a	1634 a	0.711 a	291 ab
L	***	NS	*	***	**	NS
Q	**	NS	*	*	*	NS
Genotype						
Arinda	46.3 a	651 a	2143 a	1517 a	0.686 a	287 a
Ninfa	44.0 b	654 a	2164 a	1510 a	0.692 a	293 a
Rubino	43.5 b	632 a	2144 a	1529 a	0.698 a	243 b

L=linear; Q=quadratic; * P<0.05; ** P<0.01; *** P<0.001. Relationship, tested by regression analysis, between nitrogen rate level and responses of each character.

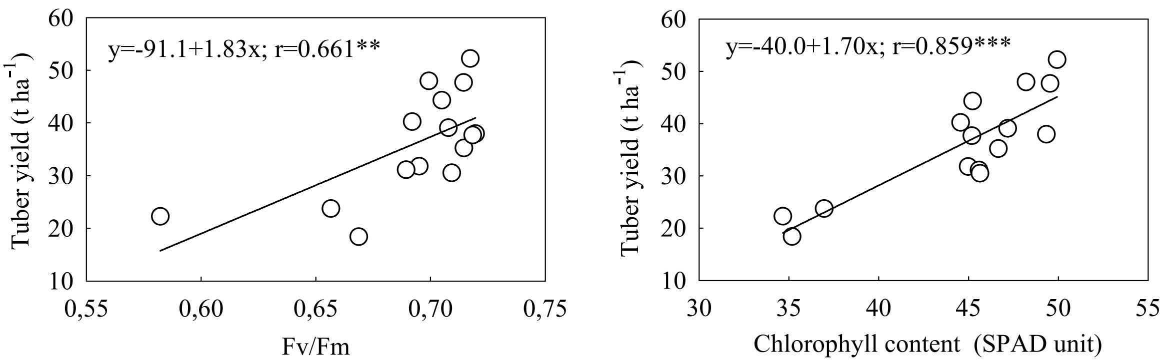

Figure 1 - Relationship between tuber yield and F_v/F_m ratio (on the left) and chlorophyll content (on the right), on average of measurement date.

In our experiment F_m, F_v/F_m and T_{max} proved to be more sensitive chlorophyll fluorescence parameters of potato plant nitrogen deficiency (as measured in contrast between fertilized and non-fertilized materials), but resulted unreliable indicators for plant N content at N fertilization rates above 100 kg ha^{-1}. Chlorophyll content and F_v proved instead to be more suitable as predictors for nitrogen status, since they were linearly associated with nitrogen fertilization rate.

The increase of F_v/F_m at N input above 100 kg ha^{-1} suggests a positive effect of high nitrogen fertilization, but it was not responsive to increases in N rate in the range of 200 to 400 kg ha^{-1}. A decrease in F_v/F_m ratio was found in maize under N deprivation (Khamis et al., 1990) and in common bean under low N supply as a consequence of increased F_o and decreased F_m values (Lima et al., 1999).

With regards to genotype, Arinda showed the highest value of chlorophyll content; this cultivar is well adapted to the Mediterranean climate, where it gives high tuber yield (Parisi et al., 2002; Ierna et al., 2005; unpublished data). On the basis of this it is reasonable to propose an association between chlorophyll content and the adaptability of a genotype to its growing environment, and its tuber yield. In this study, tuber yield was positively related with chlorophyll content and F_v/F_m ratio, in agreement with findings by other authors on other species.

In conclusion, the results of the present study indicate that chlorophyll fluorescence can be used under field conditions to detect differences in potato response to nitrogen supply. The relationships between chlorophyll fluorescence and crop yield, support the idea that some chlorophyll fluorescence parameters can be used to assess yield performance of potatoes grown in the field and in the longer run could help to reduce the excessive inputs of nitrogen in the framework of sustainable crop systems.

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FERRIC REDUCING- ANTIOXIDANT POWER OF POTATO TUBERS (*SOLANUM TUBEROSUM*) OF VARIOUS CULTIVARS DIFFERING IN BLACKSPOT SUSCEPTIBILITY

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Blackspot is an inherent vice which impair the tuber quality. The discoloration of potato flesh develops as a result of mechanical impact on the tubers through enzymatic reactions where oxygen is involved. The damaged tissue survives as cell union or it may be destructed (Radke *et al.*, 2000). A linked influx of molecular oxygen enables the formation of activated oxygen species (Rawyler *et al.* 2002) which reduce Fe (III) (Szőllősi *et al.*, 2002). Antioxidants might minimize oxidative damage and subsequently enzymatic browning. Tuber cells contain a great pool of antioxidant compounds like ascorbic acid, α- tocopherol, superoxide dismutase, and peroxidase. There is much information about single antioxidant compounds present in potatoes, but less information about their capacity due to changing tuber physiology during storage.

The aim of the present study was to determine the antioxidant capacity in the tubers of eight potato varieties with different blackspot susceptibility (Table 1, 2). The ferric reducing- antioxidant capacity (FRAP) was determined. This method bases on the reduction of the ferric tripyridyltriazin (Fe (III)- TPTZ) complex to the ferrous tripyridyltriazin (Fe (II)- TZPZ). It evaluates the chain-breaking antioxidant capacity and the ferric reducing ability of plasma at 595 nm (Szőllősi *et al.*, 2002). To get comparable results tubers with a diameter of 40 – 50 mm has been used. Two vegetation periods and following cold storage (4°C, 95 % RH) of five and eight months affected the blackspot susceptibility of the tubers as well as the concentration of antioxidants.

Table 1: Antioxidant capacity as FRAP value (mmol Fe2+ kg-1 FM) in potato tubers differing in their blackspot susceptibility (Index%) harvested 2005 and stored for five and eight months, respectively

	Index (%)			FRAP (mmol Fe^{2+} kg^{-1} FM)		
	harvest	5 month storage	8 month storage	harvest	5 month storage	8 month storage
Afra[**c]	63	33	59	0.79	1.21	1.09
Adretta[**bc]	59	46	63	0.76	1.17	1.39
Granola[**c]	59	20	39	0.79	1.32	1.49
Renate[*a]	26	4	12	1.41	1.51	1.76
Nicola[ab]	3	8	18	1.26	1.19	1.72
Lolita[c]	3	7	15	0.73	1.15	1.59
Marabel[c]	2	3	3	0.47	1.14	1.98
Gala[c]	1	0	3	1.13	0.83	1.72

. * - *significant differences in the total blackspot susceptibility between the varieties*
[a. b. c] - *different letters indicate significant differences for FRAP values (p <0.001) regardless the storage time*

Tubers of the varieties Afra, Adretta and Granola harvested 2005 and 2006 were significantly more susceptible to blackspot (> 30%) than tubers of cv. Renate (Table 1, 2). The blackspot susceptibility of cv. Renate (~ 20%) differs significantly from that of Nicola, Lolita, Marabel and Gala. In 2005 susceptible tubers (> 30%) showed after the harvest a significant lower antioxidant capacity (FRAP 0.78 mmol kg^{-1} FM) than less blackspot susceptible tubers of varieties Nicola (FRAP 1.26 mmol kg^{-1} FM) and Gala (FRAP 1.13 mmol kg^{-1} FM). However, the antioxidant capacity of tubers of less susceptible varieties Lolita and Marabel (<10%) was as high as those of tubers of susceptible varieties (> 30%). Blackspot susceptibility of the tubers as well as antioxidant capacity differed between the vegetation periods and the storage. Generally, the values showed no significant relation between blackspot susceptibility and antioxidant capacity, where the medium blackspot susceptible tubers of cv. Renate were characterized by the highest antioxidant capacity in both of vegetation periods.

Table 2: Antioxidant capacity as FRAP value (mmol Fe2+ kg-1 FM) in potato tubers differing in blackspot susceptibility (Index %) harvested 2006 and stored for five and eight month, respectively

	Index (%)			FRAP ((mmol Fe^{2+} kg^{-1} FM)		
	harvest	5 month storage	8 month storage	harvest	5 month storage	8 month storage
Adretta****b	84	96	93	2.00	0.95	0.94
Afra***c	49	44	29	1.11	0.83	0.50
Granola**c	29	80	24	0.97	1.03	0.88
Renate*a	19	26	6	1.35	1.82	1.61
Gala[b]	16	9	4	1.04	1.60	1.02
Nicola[b]	11	15	4	1.05	1.06	0.77
Marabel[bc]	8	13	7	0.72	1.21	0.88
Lolita[b]	6	22	11	1.31	1.45	1.14

*. ** - *significant differences in the total blackspot susceptibility between the varieties*
[a. b. c] - *different letters indicate significant differences for FRAP values (p <0.001) regardless the storage time*

With respect to the different varieties, the tubers were divided into six different classes of specific gravity from 1.055 kg L^{-1} to >1.095 kg L^{-1} in 0.01 increments. An increase in the blackspot susceptibility is related to the specific gravity of the tubers (Pawelzik *et al.* 2005). In 2005, the specific gravity of the tubers varied from 1.065 kg L^{-1} to 1.095 kg L^{-1}. After harvest tubers with low specific gravity of 1.065 kg L^{-1} showed significant higher antioxidant capacity (FRAP 1.15 mmol kg^{-1} FM) than those of high specific gravity of 1.095 kg L^{-1} (FRAP 0.86 mmol kg^{-1} FM). A relation between specific gravity of tubers and blackspot susceptibility at the harvest time was indicated (r^2 = 0.71). However, after five month of storage no correlation was found. The antioxidant capacity in tubers with different specific gravity is not different. After eight month storage a significant relation of both aspects is pointed out (r^2 = 0.92). Higher specific gravities implicate significantly lower antioxidant capacity. In 2006 tubers had specific gravities between 1.055 kg L^{-1} and 1.095 kg L^{-1}. In freshly harvested tubers with specific gravities <1.075 kg L^{-1} the antioxidant capacity was significant lower (FRAP ~ 1.1 mmol kg^{-1} FM) than in tubers with specific gravity <1,085 kg L^{-1} (FRAP 1.56 mmol kg^{-1} FM) and < 1.095 kg L^{-1} (1.32 mmol kg^{-1} FM). After harvest a relation between antioxidant capacity and blackspot susceptibility was not found. However, it occurred after five (r^2 = 0.81) and eight month (r^2 = 0.96) of storage. Concluding, this research indicates a relationship between the antioxidant capacity and the enzymatic blackening after a long term storage of tubers with different specific gravity. The waxy variety Renate generated higher amount of tubers with low specific gravity and was characterized by highest antioxidant capacity. The storage time affected considerable tuber physiology as well as blackspot susceptibility and antioxidant capacity. To clarify the importance of antioxidant capacity in the blackspot incidence the results of the additional growing period (from 2007) will be considered.

Acknowledgment

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THE PHYSIOLOGICAL PROCESSES AT POTATO FUNCTION OF VARIETY AND THE LEVEL OF SUPPLIES PEDOCLIMATICAL CONDITIONS FROM SOUTHERN COUNTRY AREAL

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The thermohydric stress at potato appear in summer days when the temperature is over 30^0 C and the relatively humidity is under 50%, the globally radiation is over 1000 W/m^2. This conditions has frequent in southern country. Hier, the potato cultivate the in irrigation conditions, but the stress provocated of drought maz be appear at potato for short and long time with negative effect upon production. So, we must to cause the physiological reactions of potato in this conditions function the different technological factors: The variety, the soil, irrigation method, etc.

The researches were effectuated on 6 varieties of potato, cultivated on chernozem soil and sandy soil, in irrigation conditions of 80% from IUA and unirrigated.

In the houses of big hydric deficits, the increasing traffic lights, the leaves towers, the gaseous changes has reduces, and the perspiration processes intensity is too big than intensity of photosynthesis processe. At large in, the perspiration intensity of plants on the soils with hydric deficit is less than of the plants cultivated on the soils with and what wonder moisture.

The determinations at plants were emphasized that the studied factors influenced very much upon the reaction of potato at environment conditions.

Ianosii S.(2000), He specifies that the zones with potential natural lowest is most neindicate of the culture of these potato zones presenting ecologic valuable funds, carry in conditions of conjunct irrigation and with different technological measures, can compete to the of a realization superior productions realized in traditional zones, the favorable maul.

Levitt(1980), quotation of BEUKEMA, H. P. ZAAG Van der D. E.(1990), He divides the resistance to stress in toleration and avoid. The avoiding of the stress appears if plant arrives at the equilibrium termodinamic with the factor of stress but don't appears no modification, or the which modifications appear the by-pathes remaked. In the process of the evolution, the selection he did rather to the mechanisms of avoiding, carry the by-pathes the efficient maul, than to the mechanisms of toleration, in the process of the resistance to stress.

The researches were made on 6 varieties of cultivated potatoes on soils of guys chernozems and sabulous soils, in conditions of irrigation 80% from IUA and unirrigated.

In the of a case the hidric elder deficit breed ceases, the leafs twisted, the gaseous shifts are reduced, and the intensity of the process of breath exceeds on the one the process of at large photosynthesis the intensity breath of the plants from the soils with hidric deficits is else little than one the cultivated plants on soils with the natural humidity.

They did the determinations to plants and he consisted that factors took under consideration had a very big influence about the reaction of the potato to the conditions of average.

Keywords: Photosynthesis, perspiration, variety, vacuolar juice, material water

RESULTS

The watery amount from oscillated equal-phase of button between 85 to the variety Agata and 89, 3 to the variety Rozana. He consisted a hydration foliage an erect to all the studious varieties through the watery reserve eighth from insured soil of the precipitations geted down on this aisles .

The amount of dry substance from registered next values: 13-15 to the variety Sante, 11-12 to the variety Amelia, 12-14 to the variety Tâmpa, 11-15 to the variety Agata and 10-14 to the variety Rozana.

In the climatic conditions from this stage, concentration of vacuolar was stricken more of the variety studied than of the minimum ceiling. The variety Sante and Tâmpa registered the values contained between 2, 0-2, 9, the variety Rozana between 1, 5-1, 8 and the variety registered most erect values at 4, 5-5. the which varieties presents the big values concentration of vacuolar juice is adapted else easy to conditions of stress termohidric, be else proof drought.

In fenofaza of flowery (the June) the watery amount to level foliage he maintained erect to all the studious varieties between 85-91. The amount of dry substance in oscillated between 9 to the variety Rozana and 15 to the varieties Agata and Tâmpa. The concentration of the cellular juice he differentiated between varieties, to record values all maxims to the variety Agata (5, 3-5, 6).

The hydration foliage erect in these stage influienced positive the rhythm of growth of the plants of potatoes, having a vegetative very apparatus developed.

The intensity were influienced so the climatic factors (the temperature of the air, the relative humidity, the precipitates) quotients and of the studious varieties . The variation diurnal emphasized the values contained between 8, 1-15, 0 g/ 10 g s. p. to the hour 8 the morning to all the varieties. At noon (the hour 12) the values of the perspiration bred along with the lift of the temperature of the air to 28^0 C and diminish the humidity relateing to 50%. Thus, to the variety Sante the intensity touched 23, 6 g /10 g s. p. and to the variety Agata 23, 1 g 10 g s. p. to the hour 16 the intensity of the perspiration diminished to all the varieties, be contained between 9, 2 - 18, 1 g /10 g s. p .

The intensity were accessible to the climatic conditions, the varieties studied and stages of vegetation. Thus, in stage of button they registered next values: 43-59 mg s.u./dm^2 /8 hours to the variety Sante, 58-75 mg s.u./dm^2 /8 hours to the variety Amelia, 43-75 mg s.u./dm^2/8 hours to the variety Tâmpa, 70 - 89 mg s.u./dm^2/ 8 hours to the variety Agata and 56 - 70 mg s.u./dm ^{2}/ 8 hours to the variety Rozana.

In the stage of flowery, phase of accumulations maxims to the plants of bred, the intensity of the photosynthesis again asimilatele am transported to the level of the tubers. The varieties Agata and Tâmpa registered the average daily values contained between 54-90, 5 mg s.u./dm ^{2}/ 8 hours.

The watery amount in leafs registred in the stage of were accessible to the amount of hurried geted down on this period. The values registred were contained between 89, 4 to the variety Tâmpa and 92, 9 to the variety Agata.

The amount of dry substance in oscillated between 7, 1 to the variety Agata and 10, 1 to the variety Amelia. Concentrations of vacuolar were accessible to the cultivated variety and the variant of irrigation. The values were contained between 1, 8 to the variety Tâmpa and 5, 0 to the variety Agata.

The foliage hydration erect in this permited the development in optimum at physiological process. Thus, the intensity presented a variation a diurnal, be influienced of the climatic factors and agrotechnics.

The morning, to the hour 8 the values of the perspiration at foliage were contained between 3, 2 g/ 10 g s. p./ hour to the variety Rozana and 4, 8 g/ 10 g s. p./ hour to the variety Sante.

At noon (the hour 12) with the lift of the temperature of the air to 28^o C and diminish the humidity relateing to 50%, he intensified the evaporation of the water through perspiration. The values of the perspiration were contained between 9, 5 g/ 10 g s. p./ hour to the variety Tâmpa and 14, 2 g/ 10 g s. p./ hour to the variety Tentant. Of mentioned that the varieties Agata, Tentant and Amelia registered the values maxims at intensity of the perspiration to the hour 16. The average daily values oscillated between 7, 4 g /10 g s. p./ hour to the variety Tâmpa and 11, 4 g/ 10 g s.p. /hour to the variety Tentant.

In the variant of irrigation to the minimum ceiling of 50% from I.U.A. the values intensity of the perspiration were else reduced comparative with the variant in which the humidity of the soil he maintained above ceiling of 80% from I.U.A. thus, in first case they registered next values: 2, 9-3, 5 g/ 10 g s. p./ hour to the hour 8 the morning, 8, 5-16, 5 g/ 10 g s. p./ hour to the hour 12 and 7, 4-12, 0 g/ 10 g s. p. hour to the hour 16. He consisted a big difference concerning the intensity of the foliage perspiration to the studious varieties because do the part from different from early groups. In the phase of flowery, to the plants of potatoes waves the minimum ceiling of humidity he maintained above the level of 80% from I.U.A. the values of the foliage perspiration were erect, registred to the hour 16 between 5, 0 - 17, 5 g /10 g s. p./ hour.

When the plants are good aprovizionate with water the hidric balance-sheet is positive, established an equilibrium between absorption and perspiration.

To the plants of potatoes from the variant unirrigated the values of the perspiration were else reduced, be contained between 5, 4 - 10, 4 g /10 g s. p./ hour at noon and 3, 5 - 6, 0 g/ 10 g s.p./ hour to the hour 16.

The intensity was accessible to potential genetic of the variety and the climatic specific factors of zone of culture. In the beginning the month of the June (13. 06) the values of the photosynthesis were contained between 23, 1 - 82, 9 mg s.u./dm ^{2}/ 8 hours.

Toward exhausted the month of the June registered the values contained between 50, 9 - 69, 7 mg s.u./dm ^{2}/ 8 hours to the plants waves the humidity were maintained above the level of 80 from i. In the variant unirrigated registered the negative values to the varieties Agata, Sante and Amelia, the plants below the point of compensation. The early varieties cultivated in regimes unirrigated arrived at the maturity, asimilatele from the level at foliage be translocate in tubers.

Conclusions

1. The which varieties presents the big values of concentration of vacuolar juice is adapted else easy to conditions of stress termohidric, be else proof drought.

2. The amount of dry substance in oscillated between 9 to the variety Rozana and 15 to the varieties Agata and Tâmpa.

3. Concentration the cellular juice he differentiated between varieties, registered values all maxims to the variety Agata (5, 3-5, 6).

4. The foliage hydration erect in these stages influiențat positive the rhythm of growth of the plants of potatoes, having a vegetative very apparatus developed.

5. The intensity were stricken so the climatic factors (the temperature of the air, the relative humidity, the precipitates) quotients and of the studious varieties.

6. The variation Diurnal Emphasized the values contained between 8, 1-15, 0 g/ 10 g s. p. to the hour 8 the morning to all the varieties. At noon (the hour 12) the values of the perspiration bred along with the lift of the temperature of the air to 28 ^{0} C and diminish the humidity relateing to 50%. Thus, to the variety Sante the intensity touched 23, 6 g/ 10 g s. p. and to the variety Agata 23, 1 g/ 10 g s. p. to the hour 16 the intensity of the perspiration diminished to all the varieties, be contained between 9, 2 - 18, 1 g/ 10 g s.p./hour.

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PATHOLOGY

VERTICILLIUM WILT OF POTATOES: PATHOGEN DETECTION AND INTERACTIONS

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Verticillium wilt of potato is an economically important disease that causes early death and reduced tuber yields. Chemical treatments for disease control are fast and effective, however, due to environmental, health and cost concerns non-chemical methods, such as crop type selection, crop rotation periods and land management practises, are being explored as environmentally-benign measures for reducing pathogen populations and disease problems. The purpose of this study was to determine how the different Verticillium species interact and affect the development of the host. In field and greenhouse studies, the progression of Verticillium albo-atrum, V. dahliae, V. tricorpus and V. albo-atrum (Group 2) in potato plants was determined. Plants were inoculated, through the soil, with one of the four species or a combination of two. The presence of the four pathogens was determined in various plant tissues and soil samples on a number of destructive sampling dates using Polymerase Chain Reaction (PCR) techniques. High population levels of V. dahliae in field studies suggest that it is the more aggressive pathogen while relatively low population levels of V. tricorpus and V.albo-atrum (Group 2) indicate that they are the weaker pathogens of the four studied. These results were compared with visual symptoms to obtain a better understanding of the host-pathogen relationship.

IDENTIFIED SPORES DURING POTATO POSTHARVEST.

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In this work it was carried out a control of fungal spores present into potato storage during 4 years (2002-2006).

To carry out this work we used a Lanzoni VPPS 2000 Hirst volumetric sampler placed into a potato storage, which is situated in "A Limia" NW Spain. In this region, with an average production of 5 million kg per year under the protected geographical indication (IGP) Pataca de Galicia, this label is recognized by the European Community.

During the studied period a total amount of 160000 spores was identified and included in 50 spore types. An important amount of it is non pathogenic spore types and this represents an important percentage on total identified spores.

EVALUATION OF THE DIFFERENT MILDIU PREDICTION MODELS ON A POTATO CROP IN A LIMIA NW OF SPAIN

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In this work it was carried out a control of fungal spores presented over a potato crop during 3 years.

To carry out this work we used a Lanzoni VPPS 2000 Hirst volumetric sampler placed into a potato storage, which is situated in “A Limia” NW Spain. In this region, with an average production of 100 million kg per year, the potato is the most important crop.

Different prediction models are evaluated. Smith periods and NEGFRY model is the most suitable model for this area. Negative prognosis model saw good results during the last part of culture period, but it is not effective to predict the elevated spores period during the first part of the culture.

EFFECTS OF SEED INFECTION LEVEL BY SILVER SCURF (*HELMINTHOSPORIUM SOLANI*) ON THE INFECTION LEVEL OF HARVESTED POTATOES

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Introduction

Damage by silver scurf in organic potato production is an increasing problem in The Netherlands and other N.W. European countries. Silver scurf, a blemish disease caused by Helminthosporium solani, may enhance water loss from tubers during storage, resulting in reduced tuber quality at store unloading. While the development of silver scurf (*Helminthosporium solani*) mainly takes place during the storage period, the main primary infection sources for newly harvested tubers, however, are the mother tubers planted in spring, producing silver scurf spores on existing (and expanding) lesions. These spores are transferred to the newly formed tubers by means of passive transport, or can infect the new tubers during harvest.

Silver scurf on an infected seed tuber starts to sporulate after planting. Sporulation is most intensive at the border of the lesions. Silver scurf in a growing lesion colonizes fresh skin continuously, and even on almost clean seed the amount of spores produced may be very high. When a seed tuber is completely overgrown with silver scurf, there is no 'fresh' skin on which the fungus can sporulate. The number of spores that is initially formed is thereby reduced, and the harvested daughter tubers will have less silver scurf affected surface.

This hypothesis was tested in 2006 and 2007 on two locations. Seed with a high level of silver scurf infection was planted next to seed with a low level of infection. In all experiments the more heavily infected seed resulted in cleaner tubers at harvest than the less infected seed (see figure).

Experiments

The experiment was laid out on two organic arable farms in Flevoland (The Netherlands) in 2006 and 2007 with potatoes of the variety Santé. In 2006 seed potatoes with two infection levels (low and high) were used in the experiments, in 2007 three infection levels were used (see table 1). The potatoes were planted by hand. Fertilisation and crop management were according to the management of the commercial potato crops grown on both farms.

Table 5 Seed infection levels (% skin covered by silver scurf)

	Seed infection	low	intermediate	high
2006	Farm 1	4		37
	Farm 2	6		48
2007	Farm 1	24	38	66

Silver scurf assessments

Tuber samples were taken at the harvest moment (50 tubers per plot). Tubers were dried, and stored at 4 °C until incubation. Before assessment for silver scurf, tubers were washed, and incubated for 10 days at 18 °C at a relative humidity of 90 % or more. After incubation the tubers were assessed for the percentage of the skin that was infected by silver scurf.

Results

<u>*Crop emergence*</u>

In 2007 crop emergence was negatively influenced by a high seed infection by silver scurf: in the plots with the high seed infection level, only 67 % of the tubers emerged, in the plots with the lower or intermediate seed infection level, 83 or 89 % of the tubers, respectively, emerged (figure 1). In 2006 no differences in crop emergence were detected.

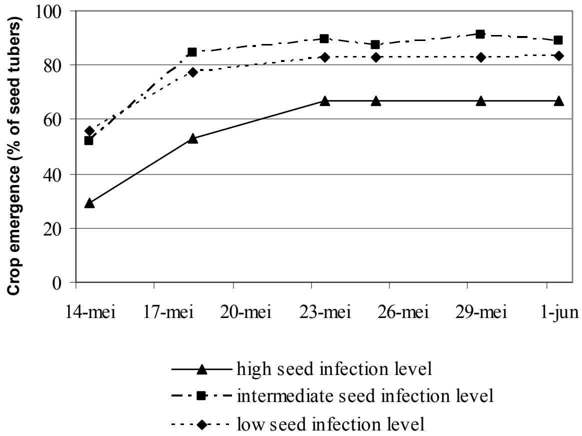

Figure 4 Crop emergence in 2007

Silver scurf

In both years a higher seed infection level resulted in cleaner tubers at harvest than a lower seed infection level (table 2).

Table 6. Silver scurf infection of daughter tubers.For comparisons within one year and one farm: values with different letters are significantly different from each other (5% level).

	Seed infection	low	intermediate	high
2006	Farm 1	4,66b		2,61a
	Farm 2	21.10b		16.90a
2007	Farm 1	0.598b	0.557b	0.118a

Discussion and conclusion

Planting potato seed that is highly infected by silver scurf may result in the harvest of cleaner tubers, with less silver scurf, than when seed with a lower infection level is used. This corresponds to personal communications from (organic) farmers who have the same experience.

Crop emergence, however, may be negatively influenced by a high infection level of potato seed by silver scurf.

ENVIRONMENTALLY-FRIENDLY CONTROL OF COMMON SCAB ON SEED TUBERS

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Common scab on potato, caused by *Streptomyces* spp., can result in severe damage to potatoes. The increased prevalence of this disease, especially in Ma'on region (South-West Israel), is attributable to several factors, among which are: susceptible crops such as peanuts and radish being grown in short rotations, soils that are conducive to the disease, and the absence of effective eradication methods. The primary inoculum source of *Streptomyces* is seed tubers. Once the pathogen is introduced and established in the soil, it survives for a long time, and only radical soil fumigation can reduce the pathogen levels. Although it is recognized that contaminated potato seed tubers are the major means of spreading the pathogen, so far any of the tested seed treatments was efficient in reducing the disease incidence or severity.

The objective of the present study was to evaluate a stabilized formulation of hydrogen peroxide agent (3.5-7% H_2O_2) as a seed treatment for controlling common scab on potatoes.

Seed borne inoculum arrives through certified seed lots imported from Northern-Europe for the spring. In average 26% of the seed lots were contaminated with intermediate and high levels of common scab between 2004 and 2007.

Seed tubers (cv. Desiree) highly contaminated with common scab were treated with SG101 or Bactoril (Quat Ammonium) by a low volume spray, and moncozeb by dusting). Two experiments arranged in a randomized complete block design, with four replications were planted in two sites (Gilat -loess soil and Halutza - sandy soil). In Gilat, the incidence of progeny tubers with russet scab was significantly reduced in both SG101 and mancozeb treatments. In Halutza, the incidences of russet and common scab were significantly lower in SG101, compared with the control and treatment s with Bactoril and mancozeb.

The findings in this study indicate the high potential of hydrogen peroxide agent as a seed treatment for controlling scab in the short-term (in the harvested progeny). Furthermore, because of this efficiency, an advantage for the long-term is being achieved, by preventing the spread of the pathogen and infestation of soils.

BIOFUMIGATION FOR REDUCING SOILBORNE PATHOGENS IN POTATO PRODUCTION SYSTEM

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Soilborne pathogens may cause severe reductions in potatoes' yield and/or damage tuber quality, resulting in serious economic losses. Pathogens such as *Rhizoctonia solani*, *Verticillium dahliae* and *Colletotrichum coccodes* are difficult to control because of their persistence in the soil and wide host range. Biofumigation refers to the suppression of soilborne pathogens and pests by volatile biocidal compounds (mainly isothiocyanates) released from decomposing Brassica tissues incorporated into the soil when used as green manure crops. We developed a bioassay in which only the volatile compounds' effects are tested, as an efficient tool for the rapid screening of plants for their biofumigation potential, and for the optimization of different aspects of the process (such as green manure concentration and most effective plant organs).

Evaluation of the effects of amendments of broccoli, rocket and mustard (30% v/v) on *R. solani* viability showed a significant reduction in *R. solani* colony diameter after 3 days of incubation. After 21 days of incubation, cabbage and turnip residues also significantly reduced fungal colony diameter. The in-vitro data indicated that all cruciferous green manure crops tested suppress *R. solani*, with broccoli, mustard and rocket being most effective.

The fungicidal effect of cruciferous crops grown and incorporated into the soil before potato crop, against these pathogens was demonstrated in field experiments: yields were increased and diseases were suppressed.

In summary, biofumigation can be considered a useful addition to the integrated pest management (IPM) arsenal for the reduction of soilborne pathogens especially in organic management, where it may be the promising solution.

PHYTOPATHOLOGICAL SITUATION ON POTATO IN BELARUS

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The control of potato diseases in Belarus is reviewed. Control of late blight (*Phytophthora infestans* (Mont.) de Bary.) has only been adequate for limited periods in the past (when resistant cultivars were available and when the systemic fungicides were introduced). Currently, the disease is in a new epiphytotic phase and control has failed again, probably connected with the introduction of new mating types and the existence of greater variability. Blight now occurs earlier in the season and in most years (instead of only years when climatic conditions are favourable). Control has now to be essentially preventive, rather than based on forecasting. The importance and control of other potato diseases is also considered (*Alternaria* spp., *Rhizoctonia solani* Kühn., *Geotrichum candidum* Link ex Pers., *Phytophthora erythroseptica* Pethyb., *Sclerotinia sclerotiorum* (Lib.) d. By), *Streptomyces spp.*).

Introduction

Production of high and stable yields of potato, and adequate storage of the harvested crop, is now a matter of increasing urgency in Belarus. Losses from pests have reached high levels in recent years. One of the main factors in this is the development of several important diseases on potato plants in the field, and on potato tubers in store, owing to a lack of cultivars with multiple resistance to plant pathogens and of effective fungicides. Moreover, in the last 10 years, the role and relative importance of individual pathogens has changed. Many widespread diseases – late blight (*Phytophthora infestans* (Mont.) de Bary.), early blight (*Alternaria solani* (Ell et Mart) J. et G., *Alternaria alternata* Keissler), scab (*Streptomyces scabies* (Thaxter) Güssow (1914) *Streptomyces griseus* (Krainsky) Waksman et Henrici, *Streptomyces globisporus* (Krassilnikov) Waksman, *Streptomyces violaceus* (Rossi-Doria) Waksman, *Streptomyces candidus* Krassilnikov, *Streptomyces chromofuscus (Preobrazhenskaya et al.) Pridham et al.*, *Streptomyces violaceoruber (Waksman et Curtis) Pridham*, *Streptomyces melanosporofaciens Arcamone et al., Polyscytalum pustulans* Owen. et Wak.), black leg (*Pectobacterium caratovorum subsp. caratovorum* (Jones, 1901), *Pectobacterium caratovorum subsp. atrosepticum* (van Hall, 1903)) – have increased in importance. Diseases previously considered as of limited incidence and only potentially dangerous (*Geotrichum candidum* Link ex Pers., *Fusarium* spp., *Phytophthora erythroseptica* Pethyb., etc.) have caused more damage to potato. After a long period of absence from potato crops, *Clavibacter michiganensis* subsp. *sepedonicus* Spieck & Kotth. has started to be detected again causing damage. This has led to a revision of existing strategies for protection of potato against fungal and bacterial pathogens and especially *P. infestans.*

Late blight of potato

Failure of control

Despite the long history of research on *P. infestans* since the time of its appearance in Europe in 1845, late blight has not become less damaging. Specific individual achievements in control of the disease, such as breeding resistant cultivars and synthesis of systemic fungicides, have to be set against long periods of failure, when damage was measured in millions of tones of lost yield. This was acknowledged in the conclusions of the Dublin Conference of 1990 on late blight and the potato famine. They recognize that the danger of late-blight epiphytotics has hardly decreased in the last 150 years. Late blight remains today the most harmful potato disease in all parts of the world, including Belarus. The situation has changed especially sharply in the last 10-15 years, when the hope for a possible victory over late blight with the help of systemic fungicides was very soon replaced by disappointment in their efficiency. Even mixtures of systemic and contact preparations have not been sufficiently successful. The second migration of *P. infestans* around the world has begun.

In our view, the basis of this failure lies in the great adaptability of the pathogen. New races of the fungus appear much faster than Man can breed resistant cultivars or synthesize new fungicides.

New features of late blight

P. infestans now infects potatoes during the whole growing season, from emergence to natural senescence. The first symptoms of the disease are now seen in Belarus 20-30 days earlier than before (first half of June) and appear simultaneously on cultivars of all maturity and resistance groups. In 2002, the disease was detected in Grodno' region on 24 May, the earliest date that it has ever been seen in Belarus.

There have also been fundamental changes in the way that blight develops on the potato plant. Previously, late blight moved from the bottom of the plant to the top. In recent years, symptoms of the disease have more often been seen first on upper leaves and stalks, and only later on middle and lower ones. Many widely grown cultivars in Belarus now show this kind of symptom on a massive scale. This new feature has made the disease more harmful, as the loss of the upper parts of the plant concerns its most functionally active tissues, affecting its efficiency much more than loss of the lower parts. It has been suggested that the stalks are infected by a special form *of P. infestans,* but this has not been confirmed experimentally. Laboratory and field experiments have shown that all infected organs of the potato plant are infected by the same form of the fungus. No particular race, or mating type, of the pathogen was associated in time with the infection of the upper stalks and leaves.

Our research has shown that the pathogen has changed its reaction to external environmental factors, especially temperature and humidity. Epiphytotics of late blight are now seen almost every year in Belarus, although the weather conditions of the growing season vary from warm and dry to cold and wet.

In the last 10 years, in the absence of control measures, losses on early cultivars at the end of season have reached 80-100%, on mid-season cultivars 70-80% and on late cultivars 60-70%. Heavy infection of potato by late blight has been observed at day temperatures higher than +30°C and night temperatures not lower than +20°C, under moisture deficiency.

Reasons for the change in late-blight severity

The main reason for this great increase in the harmfulness of late blight is a fundamental change in the structure of *P. infestans* populations. Races of the pathogen with 13 virulence genes are now detected, allowing for the possibility that over 16000 different forms occur in the country. More than 481 races were identified in 2007 in Belarus, and three mating types of *P. infestans:* A_1, A_2 and the self-fertile type A_1A_2. Isolates of A_2 type have increased in number from 1.8% in 1989 to 45.5% in 2006. This complicates the situation on potatoes because mating type A_2 is characterized by higher aggressiveness and virulence, and the ability to develop under conditions unfavorable for mating type A_1. Moreover, early appearance of the disease is also related to the possibility that mating type A_2 infects seed tubers, survives in them and, in the presence of mating type A_1, forms sexual structures (oospores), which can persist in soil for 4-5 years, and according to some authors up to 30 years.

Over recent years, potato cultivars with vertical (race-specific) resistance, or a high level of horizontal (non-race-specific, prolonged, field) resistance, have not been developed, nor cultivars combining both types of blight resistance. There have been no essential changes in varietal structure, in the technology of potato growing or in the climate of Belarus. One must therefore suppose that one of the main reasons for the rapid microevolution of *P. infestans* is the universal use of systemic fungicides against the disease without comprehensive and deep study of their after-effects on the population of the pathogen. For 3-4 years, the systemic preparations completely protected potatoes from the disease, but this gave time for adaptation of the fungus to them and for the origin of complicated more virulent races. This has been confirmed by research in the USA and other countries.

Early blight

In some years, early blight causes as much damage as late blight. Thus, in years with epiphytotics of this disease (2002 and 2004), the foliage of all widely grown potato cultivars was over 50% infected and tuber yields decreased by 25-30%. Medium-early and medium-late cultivars suffered particularly. The peculiarity of this disease is that it involves three fungi of the genus *Alternaria: A.*

solani, A. alternata and *A. tenuis*. *A. solani* mainly infects the leaves and *A. alternata* the stalks. *A. tenuis* aggravates and completes the killing of tissues caused by the first two species.

Rhizoctonia or Black Scurf

Rhizoctonia solani Kühn. is a serious danger to seed potato production in Belarus. The absence of cultivars resistant to this pathogen and of effective disinfectants for seed potatoes can lead to up to 50% of shoot death before emergence. The 'stem canker' symptom can be found on almost all plants during the growing season, while the sclerotia of the fungus (black scurf) are found on the surface of most tubers. The development of "a white leg", the fungus perfect or basidial stage - *Thanatephorus cucumeris* (Frank) Donk. on adult plants makes 50-100%.

Rubbery Rot

Of the storage rots of potato (fusariosis, phomosis, dry and wet bacterial rots), the most damaging is rubbery rot, caused by the fungus *G. candidum.* The disease was first noticed in Belarus during potato storage in 1985/1986. The disease is now widespread. In years favourable for the development of *G. candidum,* 6-8% of affected tubers may be found in some lots. The main factors favouring tuber infection and further disease development in the field are warm weather with frequent rain at the end of the growing season, use of herbicides leading to compaction and aggravation of soil structure because of a reduction in the number of inter-row treatments, high doses of nitrogen fertilizers. Increased humidity and relatively high temperature lead to reinfection of tubers in clamps and stores.

Pink Rot

Under the conditions of Belarus, *P. erythroseptica* (pink rot) is found less frequently than the previous diseases. Single tubers with symptoms of this disease are noted almost every year. Infection is favoured by the lack of oxygen in the soil, in connection with its water-logging and compaction.

Stalk Rot

In some years, in low-lying plots with heavy top growth due to application of high rates of nitrogen fertilizers, and with air temperature not increasing over 24°C in the latter half of summer, a significant spread (up to 20%) of white rot can be observed on stalks, caused by the fungus *S. sclerotiorum.*

Common Scab

In the last 10 years, common scab (*S. scabies, S. griseus, S. globisporus, S. violaceus*, *S. candidus, S. chromofuscus*, *S. violaceoruber*, *S. melanosporofaciens*) has been epiphytotic on potato tubers throughout Belarus, irrespective of weather, cultivar or cultural techniques. This is favoured, above all, by the system of agriculture accepted in Belarus. Thus, the average soil pH exceeds 6.0, and it is well known that fields with pH acidity higher than 5.5 should not be used for potato growing, especially seed potatoes. For the same reason, it is not recommended to plant potatoes after such crops as rape, barley or sugarbeet.

Plant protection situation on potato in Belarus

The changed phytopathological situation in Belarus has greatly affected the efficiency of plant protection measures applied to potatoes. To decrease the level of tuber infection by late blight, deep ridging is recommended, just before row closure. Tubers at a depth of 10-12cm are infected 5-10 times less by late blight, and at a depth of 15 cm are not infected at all. It is very important to make the ridges correctly. Broad ridges with flat tops retain water, and with it spores of *P. infestans* and other pathogens affecting the base of the plant. This creates good conditions for penetration of these pathogens into the soil and tuber infection. On narrow ridges with sharp tops, water and spores rapidly drain into the inter-rows.

For disease control, difficulties arise in determining the date for first preventive treatment of potato with fungicides against late blight and early blight, and in defining the place and role of forecasts in the system of protection. Forecasting no longer provides optimum dates for fungicide application, or number of applications, since the existing systems do not take into account the changes which have taken place in host-parasite relations and particularly in the biology of the pathogen and time of first appearance on potato.

Protection of potatoes against late blight and early blight should now be essentially preventive, i.e. aim to delay disease appearance till later in the season. The main parameters for treatment should be the time of approach of 'critical days' for the development of *P. infestans* and phenology of the host, so that the first preventive treatment of all cultivars (irrespective of maturity group) should have been done by the time of row closure. Thereafter, ware potatoes should be treated according to short-term forecasts, and seed potatoes regularly every 8-9 days. The only criterion should be the persistence of the toxic action of the product, irrespective of the forecast degree of disease development.

Long-term observations convince us that, the longer green herbage is saved on seed plots, the more danger is created for infection of tubers by late blight. So, in years of blight epiphytotics, when the foliage dies off over a short period, tuber infection does not exceed 2-7%. However, if disease development was restrained by fungicides at a level of 15-20%, tuber infection may reach 15-20%, or even 40% or more in individual plots. In the first case, the pathogen died when the potato tops were killed; in the second a certain level of infection persisted on growing foliage. Tuber contamination of this type can be eliminated only by use of modern haulm killers on seed-potato plots. The main index for such a treatment should be the level of late-blight infection and the proportion of seed tubers which have formed. The optimum recommendation is to apply a haulm killer when late-blight infection reaches 15-20% or the tubers have formed, or in any case not later than 7-8 days after the last fungicide treatment. Haulm killers do not act on the pathogen directly, but simply deprive it of living foliage on which to live. If green parts of the plant (stalks, petioles) persist, the fungus remains viable and can infect tubers.

Another element of protection against tuber infection is crop rotation, since many pathogens persist in soil or develop on other plant species. As rubber and pink rots often appear on waterlogged soils which are oxygen-deficient during tuber formation, cultural measures should ensure that the arable layer is well aerated. Stores should be kept dry, as high moisture facilitates spread of diseases, especially bacterial ones. In spring, seed potatoes should be carefully sorted to eliminate tubers which are likely to show disease symptoms in the field, as this may lead to heavy thinning of plants.

Despite the achievements of chemical protection, an important method of disease control on this crop remains the use of resistant cultivars. However, the use of this solution is becoming more and more complicated. Breeders and resistance experts have long recognized that it is not expedient to breed new cultivars with only vertical (race-specific) resistance. Horizontal (durable) resistance is more reliable. However, successes in this direction have appeared ephemeral. Parent material with horizontal resistance is lacking, and the mechanisms and conditions of expression of horizontal resistance have been insufficiently investigated. The role of environmental factors on such resistance has been underestimated, and is much higher than was supposed earlier. Long-term observations show that this type of resistance is exhibited only under conditions favourable for the growth and development of plants of a particular cultivar. Its effectiveness is reduced under all suboptimal conditions. From our point of view, breeding potatoes for resistance to fungal diseases will only be successful in those cases where there has been precise monitoring and forecasting of the intraspecific variability of the pathogen and a corresponding program of renewal of cultivars is in place.

By use of a complex of plant protection measures on potato, it is possible in Belarus, even in epiphytotic years, to decrease foliar late-blight infection from 80-90% to 5-10%, and tuber rots from 15-20% to 1-1.5%. Thus, up to 10-12t of high-quality harvested potatoes can be saved per hectare.

VALIDATION OF NEW METHODS FOR EARLY DETECTION OF POTATO SKIN SPOT

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Skin spot, caused by Polyscytalum pustulans, is a disease that can hugely reduce the market value of susceptible varieties. However, diagnosing the disease is difficult because symptoms usually appear many months after harvest. An 'eye-plug' test is currently employed by specialist laboratories to test seed but this is laborious, slow (results often take more than a week to develop) and quantification is difficult. The BPC has funded research to develop a rapid, molecular-based PCR method for detecting the pathogen to help researchers and growers determine the levels of inoculum in seed stocks. This poster describes work undertaken to validate primers and probes developed for the detection and quantification of P. pustulans.

CHLORANTRANILIPROLE (RYNAXYPYR®, CORAGEN®): A NOVEL ANTHRANILIC DIAMIDE INSECTICIDE WITH OUTSTANDING CONTROL OF COLORADO POTATO BEETLE (LEPTINOTARSA DECEMLINEATA)

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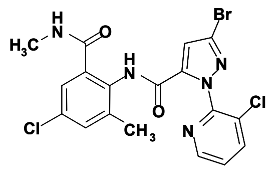
SUMMARY

Chlorantraniliprole (Rynaxypyr®, Coragen®) is a new insecticide from DuPont with a new mode of action (Ryanodine receptor modulator) that has demonstrated outstanding control of Colorado potato beetle (CPB) and no-cross resistance to any existing insecticides. The product performance on CPB in Europe is reviewed, focusing on the experimental trials carried out 2004-07 in Eastern European countries in collaboration with the local Potato Research Institutes. Besides strong efficacy, Chlorantraniliprole has a very favourable toxicological profile and minimal impact on non-target arthropods and pollinators, thus bringing potato growers a new solution for CPB control with excellent IPM and IRM fit.

PRODUCT FEATURES

Chlorantraniliprole (ISO) also known as Rynaxypyr® and DPX-E2Y45 belongs to a new chemical class of selective ryanodine receptor (RyR) agonists. Upon ingestion, chlorantraniliprole activates the release and depletion of internal calcium stores in the insect muscles. The insect rapidly stops feeding, becomes paralyzed, and ultimately dies. Differential selectivity towards insect RyRs explains the product's outstanding profile of mammalian toxicity. It is primarily active on chewing pests by ingestion and by contact, showing good ovi-larvicidal and larvicidal activity. In Colorado potato beetle, strong efficacy on adults is also observed. Inhibition of insect feeding occurs rapidly (minutes to a few hours after ingestion) and death normally occurs within 24-72 hours. In Europe, rates of 10-12.5 g a.i./ha (equivalent to 50-62.5 ml/ha of the formulated product: Coragen®) are highly effective, even on resistant CPB populations. Consistency of performance and exceptional crop safety are key product features. Whereas the new mode of action makes chlorantraniliprole a valuable option for Insect Resistance Management (IRM) strategies, safety to key beneficial arthropods and honeybees confer a strong fit within Integrated Production Management (IPM) programmes. The remarkably favorable toxicity profile combined with low use rates provides large margins of safety for consumers and agricultural workers (Bassi *et al*, 2007).

Common name:	Chlorantraniliprole
Chemical class:	Anthranilic diamide
Code number:	DPX-E2Y45
Molecular formula:	$C_{18}H_{14}BrCl_2N_5O_2$
Structural formula:	



Formulations - For use on Colorado potato beetle, Chlorantraniliprole is primarily formulated as a 20% w/v (200 g /l) suspension concentrate (Coragen®) showing good tank-stability and compatibility with conventional crop protection products.

Beneficial organisms - Chlorantraniliprole has an excellent profile of safety to beneficial arthropods and non-target organisms such as earthworms and bees and is highly compatible for use within Integrated Pest Management (IPM) programmes. The product effect on honeybees has been studied extensively (acute oral and laboratory studies and semi-field tunnel tests with *Phacelia* and wheat) demonstrating low intrinsic toxicity and high levels of safety to honey bees under field conditions. This is an important differentiating feature of Coragen® compared to most sinthethic Pyrethroid and Neonicotinoid insecticides that are currently used for CPB control.

Earthworm acute LC_{50};	>1000 mg/kg
Earthworm reproduction NOEC:	1000 mg/kg
Honeybee acute LD_{50}:	>104.1 µg/bee oral (formulated product)
	>4 µg/bee contact (sol. limit)
Wasp parasitoid (*Aphidius rhopalosiphi*) LR_{50}:	>750 g/ha
Predatory mite (*Typhlodromus pyri*) LR_{50}:	>750 g/ha

FIELD EVALUATIONS

Methodology – The European field experiments (2003-2007) were conducted following EPPO (European &

Mediterranean Plant Protection Organization) or local guidelines, in accordance with GEP (Good Experimental Practice). Treatment effects are reported as % reduction (of damage or larvae) over the untreated control using Henderson-Tilton's or Abbott's formula. The results presented here split according to two different datasets:

1) the performance of Coragen® for control of CPB larvae as a mean of all the assessments carried out season-long in 32 significant European trials (2003-06)
2) the control of CPB larvae in 19 highly significant trials from Eastern European countries that were carried out in collaboration with local Potato Research Institutions (2004-07).

PERFORMANCE IN BRIEF

The results from 32 European trials show that low rates of chlorantraniliprole (Coragen®) provided excellent control of larvae and adults of *L. decemlineata* for up to 22 days after a foliar application. 10 g a.i./ha (Coragen® at 50 ml/ha) gave better control than Pyrethoid insecticides and the same control as the Neonicotinoid standards. At the higher rate (Coragen® at 62.5 ml/ha) the product provides superior residual control, for longer than three weeks. Figure 1 represents the mean performance from all the assessments carried out 1 to 22 days after single applications.

Figure 1. Efficacy on L. decemlineata after single application. Mean of 32 tests (Europe 2003-06)

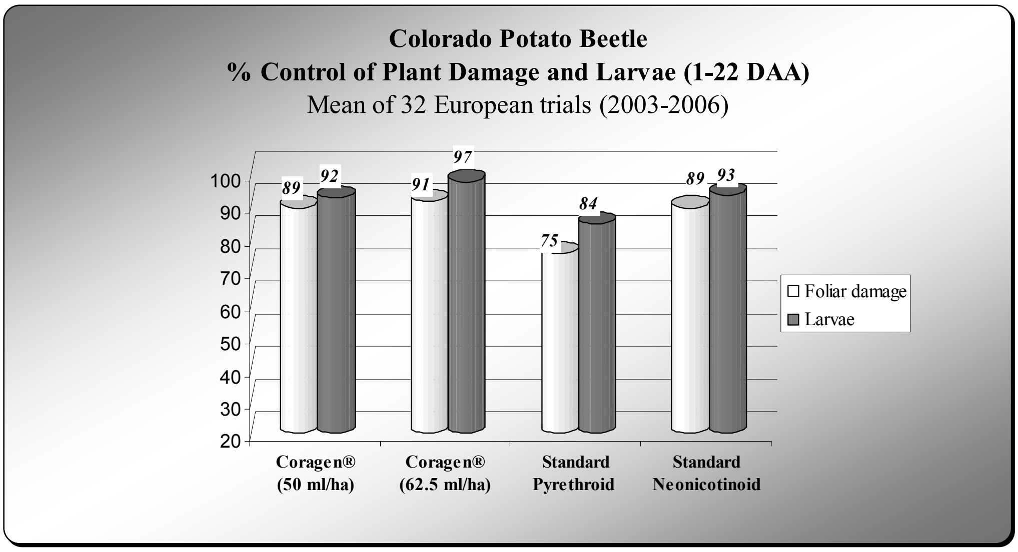
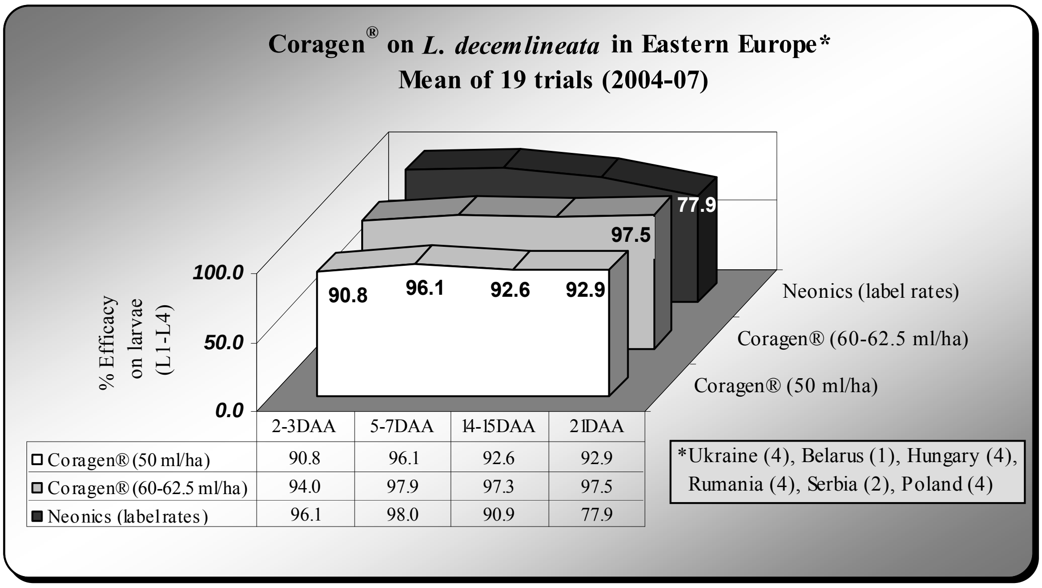
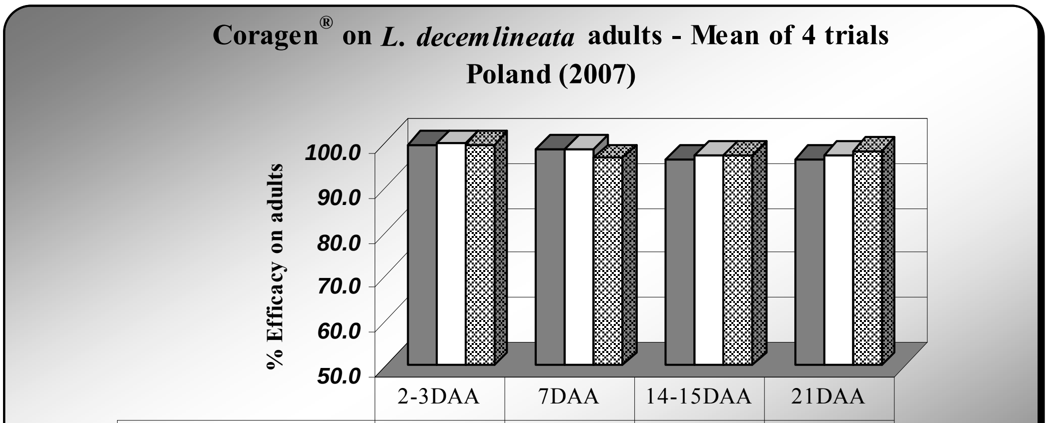


Figure 2. Efficacy on L. decemlineata larvae over time. Mean of 19 tests (Eastern Europe 2004-07)



	2-3DAA	5-7DAA	14-15DAA	21DAA
Coragen® (50 ml/ha)	90.8	96.1	92.6	92.9
Coragen® (60-62.5 ml/ha)	94.0	97.9	97.3	97.5
Neonics (label rates)	96.1	98.0	90.9	77.9

Figure 3. Efficacy on L. decemlineata adults (Henderson-Tilton). Mean of 4 tests

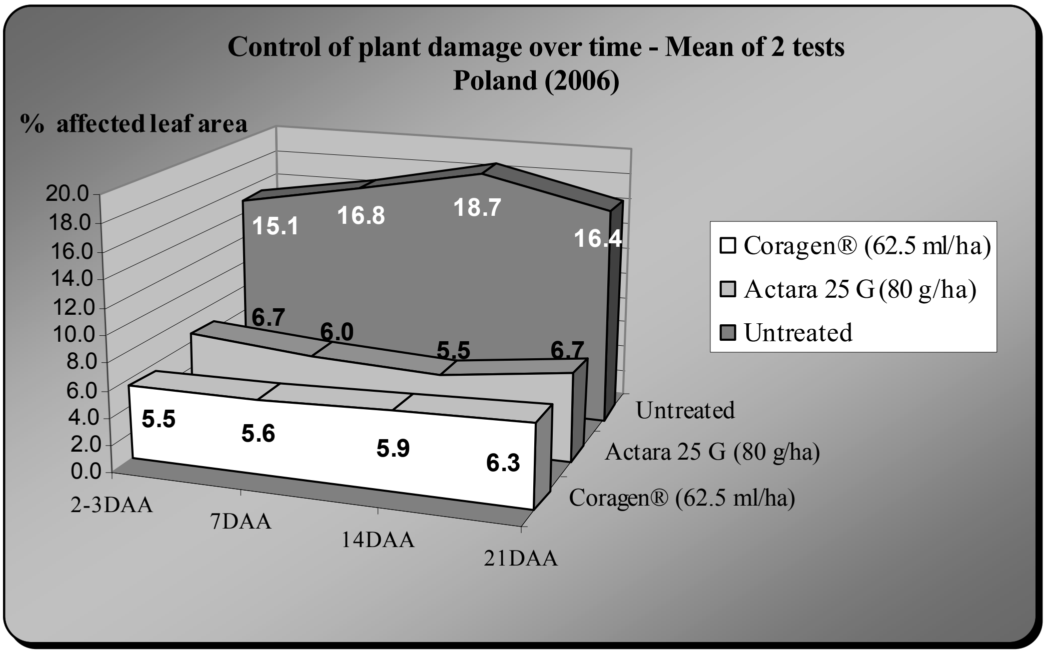


	2-3DAA	7DAA	14-15DAA	21DAA
Coragen® (50 ml/ha)	99.7	98.8	96.5	96.5
Coragen® (62.5 ml/ha)	100.0	98.9	97.6	97.5
Actara 25 G (80 g/ha)	99.9	96.8	97.4	98.5

YIELD EFFECTS

Although useful data, recording of final potato yield is not a standard requirement to demonstrate a product efficacy for the control of CPB. Efficacy is clearly demonstrated by the absence of beetle larvae and the level of crop defoliation and vigour. Potato plants can tolerate some leaf loss without significant yield reduction: for example, young plants can tolerate 20% defoliation (or loss of leaf surface) prior to flowering, but only 10% defoliation at the onset of tuber initiation. Feeding occurring within two weeks of peak flowering can have a pronounced effect on yield and this is when CPB outbreak can occur. In the field trials presented here the untreated plots in many cases were completely defoliated 30 days after application time. In a mean of 3 trials (2 from Ukraine, 1 from Belarus) the untreated plots yielded 6.1 tons/ha of tubers vs 22.4 with Coragen® at 50 ml/ha. Compared to the reference products, the crop vigour benefit provided by Coragen® was evident wherever resistant CPB populations were concerned.

Figure 4. Protection of potato plant over time. Mean of two tests with medium infestation[3].



CONCLUSIONS : EXCELLENT ACTIVITY ON CPB

The results from the European trials presented in this paper demonstrate the consistent and strong field performance of Chlorantraniliprole (Coragen®). The primary route of entry into CPB is ingestion, with secondary entry via absorption

[3] Actara is a registered trademark of Syngenta Crop Protection

through the cuticle. After exposure CPB stops feeding almost immediately, preventing further damage to plant foliage. Treated larvae initially show signs of lethargy and lack of coordination that may be severe enough to cause the larvae to fall from the plant. As demonstrated by the very low LC_{50} and LC_{90} in laboratory bioassays, Chlorantraniliprole has a very high intrinsic activity (potency) on CPB larvae, for example higher than imidacloprid. All CPB larval stages are controlled, as well as adults. An important differentiating feature of Coragen® is the long residual control provided. At the higher recommended use rates it provides extended CPB control for longer than three weeks (Tolman *et al*, 2007). Extended residue trials performed worldwide according to Good Agricultural Practices have also demonstrated no residues of Chlorantraniliprole in potato tubers at harvest. Overall the combination of strong efficacy, favourable toxicological profile and minimal impact on non-target arthropods and pollinators, means that Chlorantraniliprole (Coragen®) brings potato growers a new solution for CPB control with superior IPM and IRM fit.

CORAGEN® USE INDICATIONS

In Europe Coragen® will be recommended for use on potato crops at 50-60 ml/ha (equivalent to 10-12 g a.i./ha) with maximum two applications per crop. For best foliage protection, apply at egg-hatch or when the first damage occurs. Use the higher rate under severe pest pressure and when long residual control is sought. Use of Coragen® according to label recommendations will provide no residue in the tubers at harvest and high dietary and occupational safety margins.

ANTI-RESISTANCE STRATEGY

Chlorantraniliprole possesses a new mode of action (group 28 in the IRAC MoA classification scheme) and provides effective control of resistant CPB populations. With regards to the widespread resistance that CPB has developed to synthetic Pyrethroid and to Neonicotinoid insecticides, Coragen® should be regarded as a resistance breaker. The product will be recommended within spray programs that include rotational use of other effective insecticides with different modes of action.

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BIOFUMIGATION FOR CONTROLLING SOIL BORNE DISEASES OF POTATO *STREPTOMYCES* SPP AND *RHIZOCTONIA SOLANI*

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Because of their persistence in the soil *Streptomyces* spp. and *Rhizoctonia solani* are difficult to control. The objective of our research was to determine if this practice had a potential control activity against these diseases. We use a rapid bioassay for investigating the potential of volatile compounds of plants (*Brassica juncea*, *Sinapis alba*, *Cereal secal*) against these pathogens. Biofumigation and the effect of quantity of residues (biomass) incorporated were evaluated in greenhouse. *Brassica juncea* was the most effective in inhibiting pathogens growth *in vitro*. Incidence and severity of tuber symptoms was significantly reduced with *Brassica juncea* especially at higher biomass.

THE UNITED KINGDOM POTATO QUARANTINE UNIT: AN ACCREDITED OFF-SHORE POTATO QUARANTINE STATION FOR NEW ZEALAND

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INTRODUCTION

In 1998 the New Zealand (NZ) plant quarantine facility, Auckland, operated by the Ministry of Agriculture and Forestry (MAF) closed. Shortly after that the remaining potato quarantine facility in NZ also closed. For nearly 10 years it was not possible to import potato material for planting into NZ because the costs of setting up a quarantine facility and diagnostic laboratory to conduct the tests specified in the import health standard (IHS) were prohibitive. In 2002 NZ MAF undertook a review of the IHS for *Solanum tuberosum* tissue culture nursery stock. The draft IHS was released for consultation and the World Trade Organisation notified. Following comments, the IHS was further refined and the new IHS, issued December 2003 (MAF, 2004). The IHS lists the pests and inspection and testing requirements for MAF accredited facilities (Tables 1 and 2). Uniquely for potato quarantine requirements the IHS specifies the use of genus-specific primers for detection of viruses in the genera *Begomovirus*, *Carlavirus*, *Curtovirus* and *Potyvirus* (Table 3). The IHS allows for the accreditation of offshore quarantine facilities and diagnostic laboratories (MAF, 2001) and, following a request from a consortium of NZ growers, the United Kingdom Potato Quarantine Unit (UKPQU) at SASA applied for accreditation. The UKPQU was set up by the UK Plant Health Authorities in 1981 (originally under the name of the Breeders' Quarantine Unit) to deal with imports of potato material for planting that was prohibited under the EC Plant Health Directive, 77/93/EEC (Jeffries, 2001). Since that time, staff at the UKPQU have made significant contributions to developing potato quarantine (Brattey *et al*, 2002; Jeffries *et al*, 1993; Jeffries 1998; Jeffries & James 2005; Nisbet *et al*, 2006) and the unit continues to develop and revise its procedures, in collaboration with colleagues in the EC, EPPO and other countries (Harju *et al*, 2001), to ensure that it stays at the forefront of testing technology.

QUARANTINE TESTING PROGRAMME

The UKPQU quarantine programme at SASA is based on the EPPO post-entry quarantine procedure for potato (EPPO, 2004) using micropropagation. However, the testing is more stringent, primarily because rather than a single test on microplants or glasshouse grown plants, ELISA for viruses is conducted once on microplants and twice on glasshouse grown plants. In addition microplants are also tested for pectolytic Erwinias (*Erwinia atroseptica, E. carotovora,* and *E. chrysanthemi*). This testing exceeds the EU requirements for potato post-entry quarantine (Directive 97/46EC) and covers pests not listed in the MAF IHS standard i.e. *Ralstonia solanacearum*, *Potato leafroll virus*, *Potato virus A*, *Potato virus M*, *Potato virus S* and *Potato virus X*.

For New Zealand the additional tests required by MAF, primarily electron microscopy and the use of genus-specific primers for detection of viruses in the genera *Begomovirus*, *Carlavirus*, *Curtovirus* and *Potyvirus,* were added to the current quarantine testing programme to create a specific programme for NZ MAF. During (RT-) PCR test validation some procedures and primers recommended in the IHS were changed because of cross reactivity with healthy potato or availability of more robust tests (Table 3).

AUDITING, ACCREDITATION AND RELEASE OF LINES

The UKPQU was audited December 2006 by MAF and, after completion of test validation was accredited as an offshore potato quarantine facility on 15 November 2007. The first 17 potato lines were despatched to various potato micropropagation facilities in NZ at the end of November

2007, followed by a further 15 lines in early 2008.All lines were released with a plant health statement (Jeffries, 1998) that details the tests performed on the material.

Table 1. Viroid and viruses listed by New Zealand as quarantine pests of potato and tests required (All material must also be observed under a transmission electron microscope for virus particles)

Viroid/virus	Genus	Test	Viroid/virus	Genus	Test
Potato spindle tuber viroid	*Pos*	R or D or P	*Potato yellow dwarf virus*	*Nuc*	I
Andean potato latent virus	*Tym*	E,I	*Potato yellow mosaic virus*	*Beg*	I
Andean potato mottle virus	*Com*	E,I	Potato yellow vein virus	*Cri*	P or N
Arracacha virus B-oca strain	*Nep*	E,I	Potato yellowing virus	*Alf*	E
Beet curly top virus	*Cur*	PG	*Solanum apical leaf curling virus*	*Beg?*	V
Eggplant mottled dwarf virus	*Nuc*	I	Solanum yellows virus	*Lut*	V
Potato 14R virus	*Tob*	V	Southern potato latent virus	*Car?*	V
Potato black ringspot virus	*Nep*	E,I	*Sowbane mosaic virus*	*Sob*	I
Potato deforming mosaic virus	*Beg*	EG or PG	*Tobacco necrosis virus*	*Nec*	I
Potato latent virus	*Car*	PG	*Tobacco rattle virus* (strains not in NZ)	*Tobr*	I,P
Potato mop-top virus	*Fur*	E,I	*Tobacco streak virus*	*Ila*	I
Potato rough dwarf virus	*Car*	PG	*Tomato black ring virus*	*Nep*	E,I
Potato virus P	*Car*	PG	*Tomato infectious chlorosis virus*	*Cri*	P
Potato virus T	*Tri*	E,I	*Tomato leaf curl virus-New Delhi*	*Beg*	I
Potato virus U	*Nep*	I	*Tomato yellow leaf curl virus*	*Beg*	EG or PG
Potato virus V	*Pot*	EG or PG	*Tomato yellow mosaic virus*	*Beg*	EG or PG, I
Potato virus Y (strains not in NZ)	*Pot*	EG or PG, I	*Wild potato mosaic virus*	*Pot*	I

Table 2. Fungi and bacteria listed by New Zealand as quarantine pests of potato and tests required

Fungus	Test
Aecidium cantensis	V
Phoma andigena var. *andina*	V
Phytophthora infestans (A2 mating strain)	V
Synchytrium endobioticum	V
Bacterium	
Clavibacter michiganensis subsp. *sepedonicus*	V, IF or E, M or P, M
Erwinia carotovora subsp. *betavasculorum*	V, S
Erwinia chrysanthemi pv. *chrysanthemi*	V, S
Erwinia chrysanthemi pv. *paradisiaca*	V, S
Erwinia chrysanthemi pv. *parthenii*	V, S
Eggplant little leaf phytoplasma	PP
Potato marginal flavescence	PP
Potato phyllody phytoplasma	PP
Potato purple-top roll phytoplasma	PP
Potato purple-top wilt phytoplasma	PP
Potato round leaf phytoplasma	PP
Potato stolbur phytoplasma	PP
Potato witches' broom phytoplasma	PP

Key for tables 1 and 2 (Where more than one test is listed both are required unless otherwise indicated)

Pos, Pospiviroid; *Alf*, Alfamovirus; *Beg*, Begomovirus; *Com*, Comovirus; *Cri*, Crinivirus; *Cur*, Curtovirus; *Fur,* Furovirus; *Ila*, Ilavirus; *Lut*;Luteovirus, *Nec*, Necrovirus, *Nep*, Nepovirus; *Nuc*, Nucleorhabdovirus; *Pot*, Potyvirus; *Sob*, Sobemovirus; *Tob*, Tobamovirus; *Tobr,* Tobravirus; *Tri,* Trichovirus; *Tym*, Tymovirus;

D, Digoxigenin probe; E, pathogen specific ELISA; EG, Genus specific ELISA; I, Indicator plants; IF, immumofluorescence microscopy;M, Visual examination of Murashige and Skoog medium; N, Nucleic acid probe; P, pathogen specific PCR; PG, Virus genus specific PCR; PP, phytoplasma PCR;R, Return PAGE; S selective pectate medium; V, Growing season inspection

Table 3. Molecular tests specified in the IFS (also ELISA for geminiviruses) and alternative tests approved for use by NZ MAF following validation

Test and primer reference specified in IHS	Test and primers used by UKPQU
Carlavirus	
RT-PCR (Badge *et al*, 1996)	RT-PCR (Badge *et al*, 1996)
Potyvirus	
RT-PCR (Gibbs & Mackenzie, 1997 or Langeveld *et al*, 1991 or Pappu *et al,* 1993)	Test supplied by Jorge Abad USDA, Aphis, Beltsville, USA based on primers of Gibbs & Mackenzie, 1997 and Colinet *et al*, 1994
Begomovirus	
PCR (Rojas *et al*, 1993 or Wyatt & Brown, 1996) or universal ELISA (Agdia) for Begomoviruses	PCR (Wyatt & Brown, 1996 and universal ELISA for Geminiviruses (Neogen)
Phytoplasma	
PCR (primers fU5/rU3 (Lorenz *et al,* 1995) and primers R16F2n/R16R2 (Gundersen & Lee, 1996).	PCR primers R16F2R2 (Lee *et al*, 1993 modified by SASA) and primers fU5/rU3 (Lorenz *et al*, 1995) and primers P1/Tint (Smart *et al*, 1996 modified by SASA)
Beet curly top virus (BCTV)	
PCR (Rojas *et al*, 1993) Primers not virus specific.	BCTV specific PCR primers supplied by Jim Crosslin, USDA-ARS, Prosser, USA
Tomato infectious chlorosis virus	
RT-PCR (Li *et al,* 1998)	RT-PCR primers supplied by Bill Wintermantel, USDA-ARS, Salinas, USA
Potato yellow vein virus	
RT-PCR or hybridisation (Salazar *et al,* 2000)	Real time RT-PCR (López *et al*, 2006). Test procedure supplied by Neil Boonham, CSL, York, UK
Tobacco rattle virus	
RT-PCR (primers not specified)	Real time RT-PCR (Mumford *et al*, 2000)

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PHYTOPHTHORA

DEVELOPMENT OF INTERNET BASED ADVISORY SYSTEM FOR LATE BLIGHT CONTROL IN WIELKOPOLSKIE PROVINCE OF POLAND

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Modern Plant Protection is based on the balance between the pest threat for crops and intensification of chemical treatment. Fast delivery to the customer the information about the level of pest threat assessed by a reliable method is the basic condition for the introduction of this postulate into agricultural practice. In high developed economic countries this aim is realized with the help of Internet. Application of the Internet in plant protection is particularly useful for support decision making when genetic resistance of plants is not sufficient for pest control. Potatoes infestation by Phytophthora infestans is a good example of the situation described above. Among 122 potato varieties registered in Poland only 26 is characterized by resistance above 6 in 9 point scale. The rest needs precise chemical treatment in appropriate time. To aim this goal the Institute of Plant Protection in Poznań developed an Internet platform for decision support in potato protection against P. infestans. The user of that application gets the information about late blight threat for potato, assessed by the model of Ullrich and Schrődter and results of chosen potato plantations monitoring conducted in cooperation between Plant Protection Institute and Advisory Service.

POTATO LATE BLIGHT DISEASE: MECHANISM OF ACTION OF *PHYTOPHTHORA INFESTANS*

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Key words: potato, *Phytophthora infestans*, effectors, resistance.

Abstract

The *Phytophthora* (plant destroyer) *infestans* is the most important and devastating agent in potato crop, causing the late blight disease. Recently, the use of molecular methods in the study of *Phytophthora* such as, DNA transformation, gene silencing, genetic mapping…etc. has clarified many aspects about *Phytophthora*-plant interaction, virulence and pathogenicity, avirulence and host specificity.

To accomplish parasitic colonization, oomycetes (including *P. infestans*) use a multitude of disease effector proteins, which reprogram the defense circuitry of host cells (Huitema et al., 2004). The suppression of host defenses can occur through the production of inhibitory proteins that target host enzyme (Kamoun, 2003). Effectors are molecules that manipulate host cell structure and function, thereby facilitating infection (virulence factors or toxin) and/or triggering defense responses (avirulence factors or elicitors) (Kamoun, 2006).

In this paper, we will focus on the mechanisms of infection, the suppression of host defense response, the induction of defense response and disease-like symptoms.

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POTATO CULTIVARS FOR REDUCED INPUT PRODUCTION IN NORTHERN IRELAND

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INTRODUCTION

Late blight, caused by the Oomycete pathogen *Phytophthora infestans*, remains the most important disease problem for potato producers in Northern Ireland. For organic and reduced-input growers, who are restricted in their fungicide use, late blight has the potential to cause total crop loss. Although the most widely-grown cultivars are very susceptible to infection, cultivars with partial field resistance to blight are available. However, their response to local *P. infestans* populations must be evaluated to determine their suitability. In Northern Ireland, small-plot trials have been used to study the performance of selected potato cultivars from the breeding programmes in the UK and the Republic of Ireland. In 2006 and 2007 four cultivars were chosen for evaluation on the basis of their blight resistance. These were Santé (included for comparison as it is widely grown for organic production in the UK), Orla, which had proved moderately susceptible to foliage blight, but resistant to tuber infection in previous trials (Cooke & Little, 2004), Setanta, which had appeared highly resistant to foliage and tuber infection 2004 and 2005 (Cooke, L.R., Little, G., unpublished) and Galactica, which had not been evaluated before. Orla, Setanta and Galactica were all bred in the Republic of Ireland at Teagasc's Oak Park Research Centre: Orla is a white-skinned early, Setanta is a maincrop with a russeted red skin, while Galactica, another maincrop, has a smooth skin, parti-coloured white and red.

MATERIALS & METHODS

The trials were planted in May 2006 and May 2007 at the Agri-Food & Biosciences Institute, Belfast in a split-plot design with two fungicide regimes as main plots, four cultivars as sub-plots and four replicate blocks. The 2007 trial was planted using seed saved from each treatment in 2006 to replant the same treatments in 2007. The selected cultivars (rated for foliage and tuber blight resistance, respectively), were Santé (7, 6; rated by NIAB, UK), and Orla (4, 7), Setanta (8, 8) and Galactica (7, 4) all rated by Dowley (2007). Each sub-plot comprised two drills x ten tubers of each cultivar (3 x 1.5 m). Main plots were separated by unsprayed drills of cv. Désirée, which were inoculated (early July) with Northern Ireland isolates of *P. infestans* (A1 isolates from 2005, 2006 used in 2006, 2007, respectively) to provide an infection source. Irrigation was provided by a rain-gun when required. The treatment regimes were no fungicide and a non-systemic fungicide programme (150 g fluazinam/ha as 'Shirlan', Syngenta, 300 ml/ha). In 2006, only two applications of 'Shirlan' were made on 2 and 16 August, but in 2007, due to extreme infection pressure, 'Shirlan' was applied weekly from 20 June – 13 August (8 applications).

Foliage blight was assessed twice weekly on all drills from the time that blight was first seen in the plots until haulm destruction (6 September 2006, 27 July 2007 for untreated plots, 21 August 2007 for fluazinam-treated plots). The trials were lifted on 10 October 2006 and 13 September 2007, respectively. The yield from each plot was graded and recorded; the number and weight of blighted, soft-rotted tubers was recorded and they were then discarded. The number and weight of firm blighted tubers >35 mm was assessed (and diseased tubers discarded) in November-December and again the following January.

RESULTS

In 2006, infection developed slowly in July due to warm, dry weather and did not build up until early August. In the untreated plots, Orla developed blight most rapidly followed by Santé. By early September the untreated plots of both Orla and Santé had about 80% infection, with less blight in Galactica and Setanta (Table 1). In contrast in 2007, blight built up very rapidly in July in the untreated plots encouraged by cool, wet weather. Infection developed slightly more slowly in Santé and Setanta than in Orla and Galactica, but differences were small and by the end of July all untreated plots had over 90% infection (Table 1). In both 2006 and 2007, the plots protected by fluazinam applications were less severely attacked than the untreated plots, but many more applications were required in 2007 and even so, the final level of infection was much higher than in 2006 (Table 1). In both years, Setanta developed less foliage blight than any of the other cultivars.

Table 1. Foliage blight, tuber blight and yield of four potato cultivars, 2006 and 2007 trials

Cultivar/ treatment	Foliage blight (%)[a]	AUDPC	Tuber blight (% by number)	Tuber blight/rots (kg/plot)	Marketable yield (kg/plot)
2006					
Untreated					
Santé	86.2	765	4.27	1.48	23.2
Orla	79.4	1052	0.45	0.17	24.1
Galactica	67.5	649	2.37	0.45	19.9
Setanta	29.4	107	0.37	0.04	25.0
Fluazinam					
Santé	21.2	119	2.20	0.57	26.3
Orla	32.5	231	0.23	0.05	27.2
Galactica	14.4	114	1.22	0.50	23.7
Setanta	5.2	17	0.42	0.20	25.1
L.S.D. (P<0.05)	21.00	221.9	1.616	0.440	5.18
2007					
Untreated					
Santé	90.0	468	1.03	0.17	15.2
Orla	95.0	594	0.00	0.00	18.2
Galactica	90.0	560	1.75	0.21	15.9
Setanta	90.0	411	0.54	0.04	13.2
Fluazinam					
Santé	65.6	435	3.05	0.76	26.8
Orla	59.4	377	2.78	0.68	25.0
Galactica	56.2	514	18.79	5.70	24.4
Setanta	27.5	497	2.50	0.58	22.6
L.S.D. (P<0.05)	22.13[b]	83.0[c]	7.321	1.976	4.80

a at final assessment dates: 2006, 4 September (both treatments); 2007, 27 July (untreated), 21 August (fluazinam-treated)
b for comparison of fluazinam-treated plots on 21 August only (no significant differences between untreated plots at final assessment on 27 July).
c for comparison of untreated plot AUDPCs to 27 July only (no significant differences between fluazinam-treated plot AUDPCs to 21 August)

In 2006, Galactica yielded less than the other cultivars (Table 1), but this was related to foliar damage from the post-emergence herbicide metribuzin, which was not used in 2007. Comparing between cultivars, differences between untreated and treated marketable yields were not significant in 2006, but in 2007, the untreated plots all yielded significantly less than the treated ones, reflecting their early destruction by blight, which prevented the tubers from bulking up. Marketable yields from the fluazinam-treated plots were similar in 2006 and 2007 and there were no significant differences between cultivars. In 2006, tubers from the untreated plots developed more tuber blight than those from the treated ones. Santé had significantly more blight in tubers from both untreated and treated plots compared with the other three cultivars; Orla and Setanta developed less tuber blight than Galactica. In 2007, few blighted tubers were found in the untreated plot yield, which was not surprising since the foliage had been killed very quickly by blight, but tubers from the treated plots developed more infection and Galactica was particularly severely affected with a significantly greater percentage of blighted tubers than any other cultivar (Table 1).

DISCUSSION

These trials were exposed to a high infection pressure provided by typical Northern Ireland *P. infestans* isolates, representing genotypes which belong to the aggressive new population (Carlisle *et al.* 2002; Cooke *et al.*, 2006). In 2007, a few blight lesions were observed on one plot and on the adjacent infector row of Désirée immediately before inoculation in early July. The source of the infection is unknown: the trial site is distant from commercial crops, few outbreaks of blight had been reported in Northern Ireland by this date and none in the vicinity. Characterisation of *P. infestans* isolated from this infection revealed that it was A2 mating type, which has been rare in Northern Ireland (Cooke *et al.*,. 2006). Sampling of the trial at the end of July revealed that the vast majority of isolates obtained from foliar lesions were A2 mating type and RG57 fingerprinting (K.L. Deahl, F.M. Perez, personal communication) indicated that these belonged to an A2 genotype which has become dominant in Great Britain since 2006. Thirteen isolates from tubers (seven from Galactica, four from Santé and two from Setanta) also proved to be A2.

The greater level of foliar blight in Setanta compared with Santé in 2007 than in 2006 might indicate an interaction between blight genotype and cultivar. However, this did not appear to impact on tuber infection since Setanta developed little tuber blight in either year. Galactica tubers, however, developed very much more blight compared with Santé in 2007 than in 2006, which may also be related to the specific *P. infestans* genotypes responsible for infection. These possible interactions will be investigated further.

These trials demonstrate that although moderately blight-resistant varieties can be grown without fungicide inputs in years such as 2006, in a bad blight year such as 2007 organic crops are likely to develop foliage infection severe enough to reduce yield substantially. It is therefore very important to use a cultivar which has good tuber blight resistance as well as resistance to foliage blight. However, disease resistance is only one of the factors which influences variety choice; agronomic factors tend to be more important as are cooking and taste qualities. Blight resistance is now available in cultivars with differing appearances and maturities, offering growers a range of options.

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EXPLOITATION OF WILD AND INTERSPECIFIC DIPLOID POTATO HYBRIDS AS SOURCES OF RESISTANCE TO *PHYTOPHTHORA INFESTANS*

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Phytophthora infestans, caused late blight in potatoes, is still the serious challenge for potato breeders, in spite of the great efforts put into research it in various aspects. One of the way for improving resistance to late blight is introduction of resistance from wild relatives of *Solanum tuberosum* into pool of bred potato. Although introgression of late blight resistance from wild species is time-consuming, breeders have searched for new alleles of genes resistant to *P. infestans*. In Młochów Research Center exploitation of chosen group of wild species and diploid interspecific hybrids in case of resistance to late blight has been performed for long time (Jakuczun and Wasilewicz-Flis 2004). The most promising sources are included into genetic and breeding research.

Materials and methods

The objectives of study were 50 diploid hybrids with resistant sources from *S. stenotomum-S. phureja, S. microdontum and S. verrucosum* (Table 1) and 97 clones selected from 16 wild species listed in Table 2. Their resistance was evaluated in leaflet and tuber slices assay in scale from 1-9, where 9 = highly resistant. Pollen fertility and presence of big pollen grains were checked for all wild accessions.

Four populations (07-1, 07-2, 07-3, 07-4) from crosses between dihaploids of cultivars and accessions of *S. kurtzianum* and *S. ruiz-ceballosii* were chosen for study usefulness of this species as donors of resistance (Table 3). For greenhouse grown seedlings the segregation of resistance to *P. infestans* was evaluated with standard leaflet test (Table 3). Pollen fertility of tested progenies was checked.

Results

Advanced diploid hybrids with mixed sources of resistance to late blight from *S. stenotomum-S. phureja, S. microdontum and S. verrucosum* were obtained in recombination breeding performed in Młochów since more than 30 years. They represent high level of resistance (Table 1). The big group of diploids having in the origin *S. stenotomum-S. phureja* carrying gene *Rpi-phu1* expressed the highest level of resistance. The resistance of diploid hybrids of various resistance sources was weaker comparing to the first one. Resistance to late blight of some diploid hybrids produced 2*n* pollen grains was transferred into tetraploid level in the interploidy crosses (Jakuczun and Wasilewicz-Flis 2006).

The results indicated clones resistant in leaves and/or slices to *P. infestans* in 14 out of 16 tested accessions (Table 2). The most promising sources of resistance seemed to be *S. michoacanum*, *S. pinnatisectum* and *S. ruiz-ceballosii*. *S. ruiz-ceballosii* (2EBN) can be directly used in sexual crosses to *S. tuberosum*, while *S. michoacanum* and *S. pinnatisectum* are not crossable as 1EBN. *S. kurtzianum* was selected among accessions with single resistant clones for detailed study. This species has not been exploited as source of resistance to late blight. Clones of *S. ruiz-ceballosii* and *S. kurtzianum* were used in crosses as donors of resistance to *P. infestans*.

Table 1. Resistance to P. infestans in leaflets and slices tests in diploid interspecific hybrids.

Sources of resistance	Resistance to *P. infestans* (1-9, 9=highly resistant) in					
	leaflets test		number of tested clones	slices test		number of tested clones
	mean	range		mean	range	
stn-phu, phu	8,8	8,3 - 9	23	8,9	8,3 - 9	15
mcd, ver	7,3	6,6 - 7,9	2	6,5	4,8 - 8,1	2
stn-phu, phu, mcd, ver	8,6	6,9 - 9	18	8,1	1,6 - 9	18
mix of other sources	7,6	5,8 - 9	7	5,2	4,9 - 9	5

Table 2. Resistance to P. infestans in wild diploid species.

Species [1)]	Resistance to *P. infestans* 1-9 [2)]						Number of clones resistant in leaves and tubers
	leaflet test		number of tested clones	slice test		number of tested clones	
	mean	range		mean	range		
S. berthaultii	4,9	3,9 – 5,7	3	2,5	1,5 - 4,1	3	-
S. chacoense	4,0	2,3 – 7,8	9	8,2	6,1 - 9	8	1
S. famatinae	6,1		1	8,3		1	1
S. garsiae	6,6	6,4 – 6,8	2	7,6	7,1 - 8,0	2	2
S. gibberulosum	6,3		1	9		1	1
S. kurtzianum	3,3	1,7 – 6,4	25	5,0	1,6 - 9	27	3
S. latisectum	4,2		1	5,3		1	-
S. leptophyes	5,1		1	7,5		1	-
S. michoacanum	5,6	3,5 – 7,8	13	8,2	7,5 - 9	14	6
S. okadae	8,2		1	9		1	1
S. parodii	4,4	4,2 – 4,7	3	7,5	5,7 - 9	3	-
S. pinnatisectum	5,2	1,0 – 8,9	22	8,5	5,0 - 9	23	7
S. ruiz-ceballosii	8,9	8,9 - 9	8	8,7	7,6 - 9	8	8
S. simplicifolium	9		1	2,0		1	-
S. sparsipilum	5,2	3,8 – 6,6	2	5,3		1	-
S. vernei	7,2	7,2 – 7,2	2	4,3	4,0 – 4,6	2	-

1) All accessions are from VIR collection (Russia), except for *S. pinnatisectum* which is both from VIR and PI collections (USA); 2) scale 1-9, where 9 = highly resistant

Four seed combinations from 2006 were tested for resistance to late blight in leaflet test (Table 3). Mean resistance in following populations 07-1, 07-2, 07-3 and 07-4 were 4,4 (range 1-9), 5,3 (range 1-9), 1,7 (range 1-4,1) and 1,9 (range 1-5,1). Twenty and 44 individuals from population 07-1 and 07-2 were classified as highly resistant in leaflets. Two remaining populations (progenies of *S. kurtzianum)* were susceptible. Only progenies of *S. ruiz-ceballosii* expressed resistant individuals. In addition *S. ruiz-ceballosii* progenies possessed fertile pollen, which make possible their utilization in crosses in breeding and research.

Table 3. Distribution of resistance to P. infestans in four pot seedlings grown in greenhouse in 2007.

Population	Origin	Number of		Classes of resistance to *P. infestans* [1)]		
		planted seedlings	tested plants in leaflet test	1-4	5-7	8-9
07-1	dH Balbina x *S. ruiz-ceballosii*1	89	82	52	10	20
07-2	dH Balbina x *S. ruiz-ceballosii* 2	136	132	65	23	44
07-3	dH Kuba x *S. kurtzianum* 1	93	91	91	-	-
07-4	dH Kuba x *S. kurtzianum* 2	100	99	98	1	-

1) scale 1-9, where 9 = highly resistant

Acknowledgements

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INFINITO – A NEW STANDARD FOR CONTROLLING LATE BLIHT (*PHYTOPHTHORA INFESTANS*) IN POTATOES CROPS

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Key words: potato late blight, prevent & controlling, Infinito.

Late blight of potato *(Solanum tuberosum L.),* caused by infection of leaves, stems and tubers is the most important potato disease. The first report about potato late blight comes from Belgium in June 1845 and after then disease rapidly was spread.

Late blight is considered a re-emerging disease and has reached epidemic proportions in North America and Europe due to the development of resistance to the fungicide metalaxyl in populations of the pathogen and the widespread occurrence of new genotypes. The pathogen causes a destructive foliar blight and also infects potato tubers and tomato fruit under cool, moist conditions.

In Romania, *Phytophthora infestans* is regarded as a high-risk pathogen regarding the risk of resistance development. Taking in account the possibility of manifestation of this disease due to evolution of climatic condition the risk of apparition is very high.

Potato late blight can be successfully controlled by a combination of sanitary measures, resistant varieties and fungicide application. The fungicide applications have shown good results to protect potato foliage and tubers from late blight.

Bayer CropScience has a lot of solutions for prevention and controlling this important disease in potatoes crops. In our portfolio are included contact, systemic and translaminar fungicides such as Antracol 70 WP, Consento 450 SC, Totoo C, Melody Compact 49 WG, Secure and Infinito 687,5 SC.

The Infinito 687,5 SC fungicide, launched in 2007, has many properties such as: new mode of action with unique fungicidal properties; strong translaminar and anti-sporulant activity; consistent, high-level of disease control on stem, leaves and tubers; long-lasting activity; yield enhancing and excellent crop safety profile

CLIMATIC CHANGES AND THE POTATO LATE BLIGHT BEHAVIOR

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ABSTRACT

Potato late blight is recognized all over the world as a major limiting factor in the production. An experimental program was conducted in Brasov, Romania during 2005-2007. A control scheme was carry out beginning fungicide application early, preventive and in concordance with a good prediction of the epidemic.

The changes observed were associated with a climate very favorable in 2005, when the outbreak of the epidemic began earlier than usual, unlike 2007 when the growing season was very hot and dry.

INTRODUCTION

Late blight caused by *Phytophthora infestans* is the most destructive disease of potato crops, causing important yield losses.

Effective fungicides are required to ensure quantity and quality of yield. Unfortunately, none of the fungicides available today give absolute control of *Phytophthora*.

The fungicides must to be effectivness against rainfalls

The climatic conditions during the blight infection period are very important.

MATERIALS AND METHODS

The spraying volume was equivalent to 300 l/ha. In all trials the first application was applied at the same time when the foliage is closing the rows.

Plots are assessed for the extent of blight spots on the leaves. Each plot is assessed as a whole for percentage disease severity using a standard accepted severity key, e.g. Anonymous 1947 (Anonymous).

Table 1. Braşov – Rainfalls (mm/m²)

Period	2005			2006			2007		
	June	July	August	June	July	August	June	July	August
Monthly total	121,8	119,7	162,0	65,4	54,0	163,2	22.2	43.7	152.1
Mean multiyear	91,9	87,4	67,3	96,7	99,8	67,3	96.7	99.8	67.3
Difference	+29,9	+32,2	+94,7	-31,3	-45,8	+95,9	-74.5	-56.1	+84.8

Potato production answer very well to repeated and frequent fungicide applications knowing that almost potato cultivars are susceptible to blight (Louise Cooke, L.J. Dowley, G. Little & E.O'Sullivan, 1996)

Treatments were made in:

2005 – 14.06; 21.06; 30.06; 12.07; 22.07; 5.08; 16.08
2006 – 27.06; 17.07; 4.08; 24.08
2007 – 9.07; 16.07; 28.08; 7.09

Observations:

2005 – 28.06; 20.7; 14..08
2006 – 5.07; 15.07; 23.08
2007 - 27.08; 6.09

RESULTS AND DISCUSSION

Table2. Late blight (Phytophthora infestans) apparition and manifestation in N.I.R.D.P.S.B. Brasov experimental field during 2005-2007

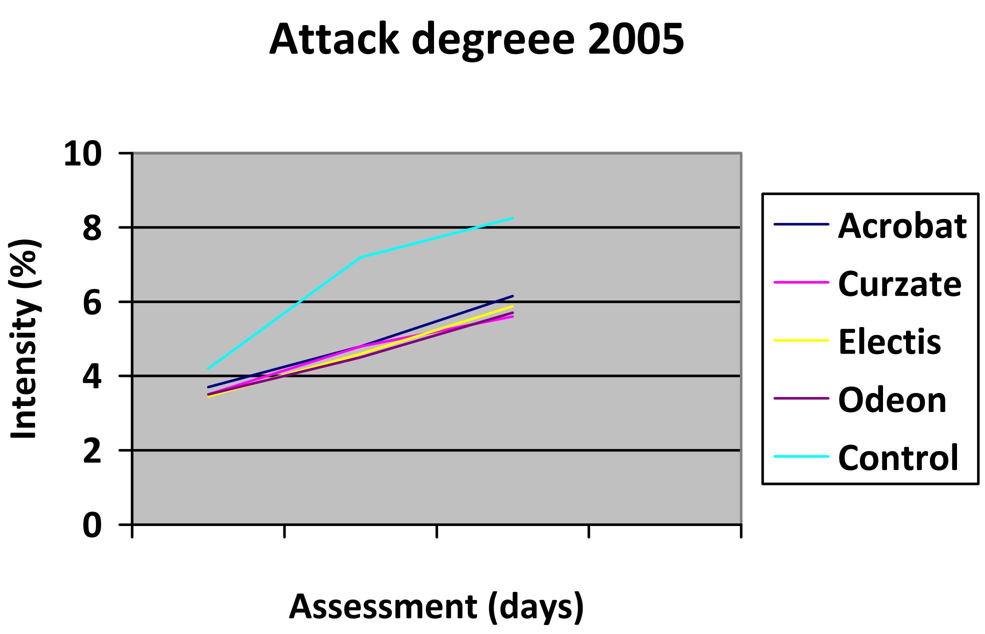
Year		Manifestation	Observations
2007	20 August	Slow evolution	Very hot summer, dry season
2006	21 June	Medium – tubers with blight	Favorable conditions for plants and pathogen development
2005	6 June	Early coming out, pronounced defoliation process - high tubers contamination	Lot of rainfalls and high termic regim

The year 2005 was an unprecedent climatic year, with a lot of rain and high temperatures all the growing potatoes season.

In such climatic condition late blight appear very early (6th June in Brasov area) and had a strong capacity of development. In one month the epidemic phase it was all over the fields.

The tuber contamination was relatively high, but specifics for a year with epidemic level of late blight.

Figure 1.

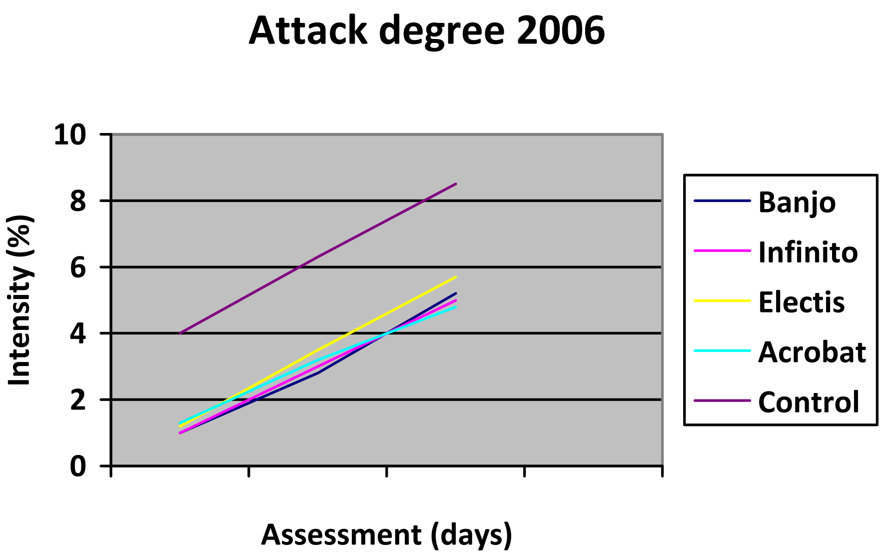


Year 2006 - Late blight first appears in 21st of June. July was very dry, without rainfalls and with 3 treatments till 15th July the infection was stopped.

Late blight was present again on the middle of August with strong capacity of sporulation all over the field.

The observations made to the tubers (18 September) showed that the contamination was relatively high, but specifics for a year with high pressure of disease.

Figure 2.

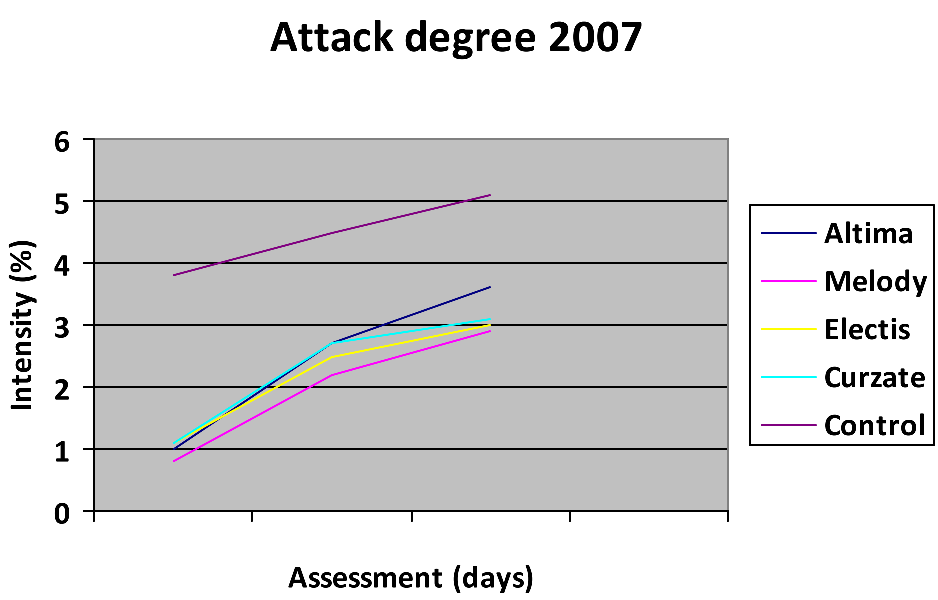


In the specific climatic conditions of the year 2007 in Braşov area late blight attack occurred in 20 August and developed slowly due to the high temperature conditions.

The observation performed (3 October) on tubers showed a low ratio of contamination.

In all years the treated leaves had a significantly higher protection than the leaves untreated.

Figure 3.



We mentioned that the tuber resistence to late blight is not corelated with the resistence to the foliage.

The frequency of tubers with late blight is directly linked with the lennght of the period of fungus sporulation.

A varieties with foliage resistence can have many damage tubers than a sensitive variety which is rapidely destroid.

CONCLUSIONS:

Climatic conditions have a powerful influence on Phytophthora infestans attack.

Mycelium survivor in tubers or soil is the results of farmers mistakes in vegetation or harvest period.

The diminish consum of pesticides to potato is possible by establish an efficient treatments program.

Fungicides have to be sprayed before the disease is present. The number of sprays can be reduced
somewhat with the use of blight forecasting, more so if the forecasts are made at the individual
farm level.

The strategy efficiency must be completed by choosing resistant varieties to blight attack.

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THE POTATO PHYSICAL AND SEQUENCE MAP AND ITS APPLICATION FOR TUBER LATE BLIGHT RESISTANCE IN SOLANUM

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Little is known about tuber resistance to *Phytophthora infestans* in potato. Most emphasis is given to foliar late blight resistance. Tuber resistance is an agriculturally important trait as infected tubers can be the source of primary inoculum once planted. Toxpeus (Toxopeus 1961) reported that important barriers to prevent the pathogen from penetrating the tuber were the tuber skin and the cambial layer just below the tuber skin. According to Flier and coworkers (Flier et al. 2001), tuber resistance could be attributed to three major components of the tuber: the epiderm, the outer cortex cell layers and the medulla. Specific necrosis, similar to the hypersensitive response (HR), was observed in the outermost 20-30 cell layers of the cortex. This necrosis involves many more cells than the HR (Pathak 1987). Moreover, potato roots (Fehrmann and Dimond 1967) and vascular regions of potato stems and tubers (Kassim et al., 1976) were found to be significantly more resistant to *P. infestans* than the other parts of the plant. Recently, *Rpi-abpt*, *R1*, *R1-like* and *Rpi-phu1* (Park et al. 2005; Sliwka et al. 2006) conferring resistance to *P. infestans* were shown to be functional in leaves and tubers. Millett and Bradeen (San Diego 2005) investigated the specificity of the *RB* gene to see whether it could confer tuber resistance or not. The *RB* gene was found to be constitutively expressed in leaf and tuber but was only functional in the leaves.

R genes from the wild *Solanum* species *S. berthaulthii, S. edinense, S. okadae, S. stoloniferum, S. chacoense, S. hougassii and S. huancabambense* were introgressed in 2X and 4X *S. tuberosum* breeding clones in order to produce tubers. Using a wounded tuber assay, it appeared that tubers from *S. edinense, S. hougassii* and *S. huancabambense* lines were fully susceptible to *P. infestans* whereas those from *S. okadae* and *S. stoloniferum* were fully resistant. More over, *S. okadae* clones displayed the same resistance spectrum in leaves and tubers suggesting that *Rpi-oka1* confers both foliar and tuber resistance. The *S. berthaultii* and *S. chacoense* clones showed variable tuber resistance responses between the different backgrounds used to introgress the *R* genes. For all the genotypes tested, the next step will be to check whether there is a correlation between foliar and tuber resistance in segergatimng F1 populations.

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BACTERIAL DESEASES

FATE OF SOIL INFESTATION BY *RALSTONIA SOLANACEARUM* OVER TIME

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Bacterial wilt, caused by *Ralstonia solanacearum*, is a vascular disease which affects more than 250 plant species worldwide, including agronomically important hosts such as tomato, potato, tobacco, peanut, or banana (Hayward, 1994). *R. solanacearum* is a soil borne bacterial pathogen, typical of tropical regions; it was not considered as a problem on potatoes in temperate regions until the appearance of cases of brown rot in several European countries in the mid 1990s.
In Europe, this pathogen is listed on the A2 quarantine list. That's why phytosanitary measures are applied when and where an outbreak is notified. For potato growers, this involves the destruction of the tubers, disinfection of storehouses and agricultural equipment, and the prohibition of potato cultivation on infected fields for the five years following the outbreak.
The aim of this study was to evaluate the capacity of *Ralstonia solanacearum* to survive in the soil, the roots of several crops of the rotation and the water systems during 8 years after a single outbreak.

Material and methods:

➡ **Description of the field and experimental layout :** the field was declared as contaminated in 2000, after the discovery of brown rot symptoms on potatoes. In 2001, the field was in fallow. Trials started in 2002. In this purpose, the plot was divided into 11 contiguous strips of 3 x 250 m; each strip was split up into 5 subplots (3 x 50 m) (Figure 1). Three aquatic areas were present on this field: one in the middle of the plot (field pond), one in the back of the plot (back pond) and the last one the road (roadside pond).

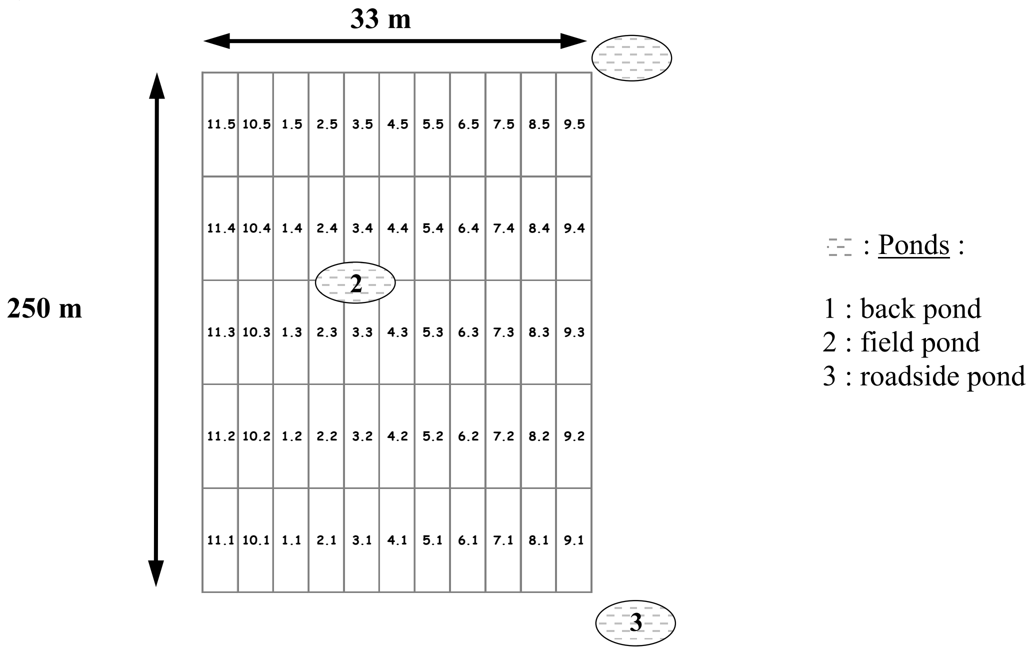

Figure 1 : Schematic layout of the experimental field and plots

For six years starting in 2002, each strip was subjected to different successions of crops, involving potato, maize, flax, barley, rye, pea, beet, rape, carrot, ray-grass and Phacelia. The succession of crops in each strip during the experiment is indicated in Table 1.

***Table 1* : Succession of crops in each strip during the experiment**

Year \ Strip	11	10	1	2	3	4	5	6	7	8	9
2002	NC	NC	Flax	Rye	Pea	Beet	Potato	Fallow	NC	NC	NC
2003	NC	Potato	NC	Beet	Rape	Pea	Barley	Maize	Fallow	Potao	NC
2004	Barley		Flax, maïze and carrot around the pond				Ray-grass		Maize		Fallow
2005	Maize		Potato		Barley		Potato		Phacelia		Fallow
2006	Potato										
2007	Groundkeepers										

NC = no crop

➨ **Samples analyses:** The soil, plants (roots and stem) and the water from the three ponds were analysed for the presence of *Ralstonia solanacearum*.

a/ Soil analyses : analyses were carried out on samples of 20 g of soil taken from the 0-10 cm layer in the subplots of the strips 1 to 6. The soil was homogenized in a TENPP buffer and mixed with a waring blender according to Poussier (2000). Total DNA was extracted using the QIAamp DNA Kit (Qiagen) and amplification by PCR was carried out using two sets of specific primers: D2-B (Boudazin *et al*., 1999) and Y2-OLI1 (Seal *et al*., 1993). Detection of the bacteria was also attempted by direct isolation on SMSA medium (Elphinstone *et al*., 1996).

b/ Plant analyses : 30 plants from each subplot were collected and tested for the presence of the bacterium. The plants were washed and superficially disinfected by rapid dipping in 70 % ethanol. The roots and/or the stem of the plant were cut into small pieces and macerated in phosphate buffer for 4 hours. Immunofluorescence (IF) and DNA extraction were performed on the macerates. The primer sets used for the amplification by PCR were the same as for the analysis of soil.

c/ Water analyses : One litre samples of the water collected in each of the ponds were filtered through a filter of 0.22 µM (Stericup Express PLUS Membrane, Millipore). Then, the filter was cut into small parts and washed in 4 ml of sterile water. This step allows a 250 fold concentration of the inoculum. *R. solanacearum* presence in the rinsing water was checked by IF, PCR and isolation on SMSA medium.

Results and discussion:

✓ **The soil**:

Analysis carried out on soil in June 2002 showed a high level of contamination by *Ralstonia solanacearum* two months after the installation of the different crops. The concentration of the bacterium in the plot varied between 10^3 to 10^5 cfu per g of soil (Fig. 2). The highest concentrations of the pathogen were located near the field pond. The inoculum decreased rapidly during the season. In 2003 to 2004, the bacterium was not detected in the soil, even in the subplots with the highest moisture.

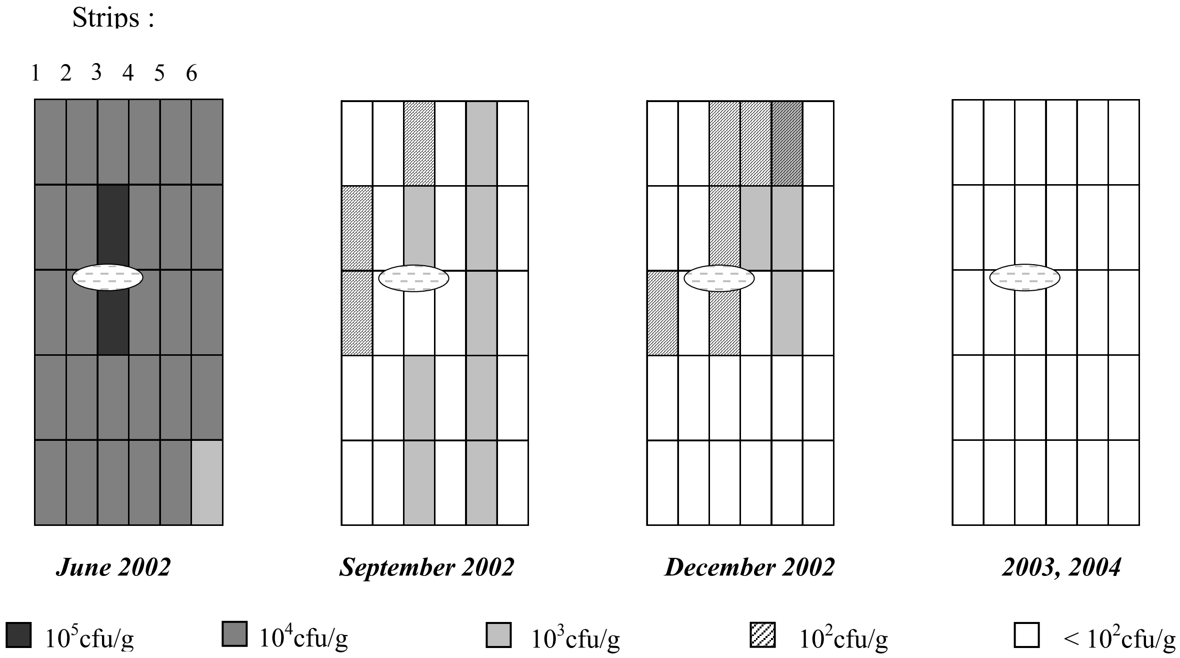

***Figure 2** : Changes in soil contamination over successive years in a plot naturally infected byRalstonia solanacearum*

✓ **The crops:**

In 2002, all the roots and the stems of the different crops tested negative. However, analyses of the soil attached to the roots were positive for some samples. In 2003, *R. solanacearum* was detected in beet, rape, pea, barley and potato plants. Tests realised on groundkeepers in subplots 2.1, 1.2, 4.1 and 5.2 also showed the persistence of the bacterium. From 2004 to 2007, the bacterium was detected in none of the different crops and groundkeepers.

✓ **The water :**

Analyses carried out in 2002 showed a contamination of the three ponds. In 2003, the bacterium was found only in the back pond, but no analysis was done for the field pond which dried out during this hot summer. Contamination of the back pond persisted through 2004, but could not be detected in either 2005 or 2006. The concentration of the inoculum was between 3.10^1 and 2.10^3 cfu per litre of water. The bacteria found were phenotype conversion mutant forms (PC forms) as described by Poussier *et al* (2005).

Conclusion :

This study confirmed the decrease of the bacterium *Ralstonia solanacearum* in soil notably in the upper layers of the soil. It is important to note that the detection was done on the first 10 cm of the soil. That's why these results cannot give information on the survival of the bacterium in the deeper layers of soil.
Moisture has a strong impact on the persistence of the bacterium in soil; therefore, it is possible that the dry summer of 2003 accelerated inoculum decline in this field.

Ralstonia solanacearum can survive in the water for several years, principally in its PC form and on the surface of plant leaves. From the present experiment, it is not possible to recommend a crop to cultivate on a plot contaminated with *Ralstonia solanacearum*, although the results suggest that pea is not a favourable crop to decrease the inoculum level in a contaminated plot. Further experiments, outdoor or in the greenhouse, are now necessary to investigate this.

Banning potato cultivation for five years after an outbreak of brown rot seems to be adequate in the pedoclimatic conditions found in this trial. Indeed, the bacterium was not detected anymore in the soil, the water and the different crops 4 years after the epidemic.

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NEMATODES

COMMERCIAL AND TECHNICAL DEVELOPMENT OF THE TRAP CROP *SOLANUM SISYMBRIIFOLIUM* FOR THE CONTROL OF POTATO CYST NEMATODE IN THE UK

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In the UK, *Solanum sisymbriifolium* has been commercially available as a trap crop for Potato Cyst Nematode (PCN) for 3 seasons. On-going, commercially funded research and trials continue to develop the agronomy of the crop. The poster describes the previous success and the current agronomic status of the crop in the UK.

As an alternative method to pesticides for reducing numbers of PCN to manageable levels, trap cropping with a sacrificial host crop is an obvious approach. Although the technique has proved very successful with potatoes as the sacrificial crop, it brings with it a high cost and obvious risk of multiplication, if not managed within tight constraints. A major advance has been the identification of *Solanum sisymbriifolium* as an alternative trap crop that, although effectively causing PCN to hatch, is fully resistant. More than 250 ha of *S. sisymbriifolium* were planted in 2007 with seed supplied by companies such as Branston Ltd under the trade name Foil-sis™.

It is an effective control technique and there is an ongoing program of research and development, with focus on the agronomy of the crop. Two particular areas have been the crop's recommended three month growing period and the requirement for soil temperatures above 12 ^{0}C. This has meant a May planting as most suitable. The derogation to grow the crop on set-aside was ideal but with the removal of compulsory set-aside and the greatly improved prices for cereals, growers are finding it difficult to include *S. sisymbriifolium* in a conventional rotation. A new seed treatment has considerably improved the germination which has helped reduce the crop duration but there is continuing research into late summer planting to follow early harvested peas or cereals.

More recently, two factors may contribute to the flexibility of the growing season. Preliminary work undertaken at Rothamsted in 2003 had indicated that a well grown and long duration crop was not necessary to have an effect on PCN. Recent studies, have also demonstrated this aspect. Perhaps more importantly, it may be the case that even at levels of exposure, too low to cause immediate nematode hatch, the eggs will be predisposed to hatch more readily at the next exposure. This could be either a second planting of *S. sisymbriifolium* or the subsequent crop of potatoes. If it is the latter, the granular nematicide applied at planting will be considerably more effective as the hatch of the PCN will be more immediate thereby exposing them to greater levels of active chemical in the soil.

THE USE OF *PHASMARHABDITIS HERMAPHRODITA* AS A BIOLOGICAL CONTROL OF SLUGS IN POTATOES

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Phasmarhabditis hermaphrodita, the mollusc specific nematode, is widely used as a biological control of slugs in salad and brassica crops. The poster describes the successful collaboration between Becker Underwood UK Ltd and Branston Ltd to develop the nematode as a non-chemical control of slugs in potatoes.

Potato crops are attacked by various species of slugs that can be found on both the surface and throughout the soil profile, with the prevailing conditions dictating the level and zone of activity. An ideal IPM strategy combines both a molluscicide pellet applied to the surface and parasitic nematodes occupying the soil profile.

New devices and methods for application of the nematodes through different irrigation systems are described as part of the successful, on-going development.

INSECTS

POTATO PLANT ACCEPTANCE BY THE PEA APHID *ACYRTHOSIPHON PISUM*

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In Europe, the most efficient vectors of phytoviruses in potato plants are the green peach aphid, *Myzus persicae* (Sulzer) and the potato aphid *Macrosiphum euphorbiae* Thomas. Nevertheless, these 2 pest species are sometimes absent or present at very low densities in traps put in non-persistent virus infected potato fields. An hypothesis has been proposed to explain virus spreading in absence of *M. persicae* and *M. euphorbiae*. Non-persistent phytoviruses are transmitted during superficial brief probes, and could be transmitted by alates of other aphid species that are not specific to *Solanum sp.* Therefore, we conducted laboratory experiments with the pea aphid *Acyrthosiphum pisum* (Harr.), which is generally reported as not feeding on potato plants but also regularly trapped in potato plots. We investigated its plant acceptance behaviour on potato plant and compared it to that of *M. persicae* and *M. euphorbiae*.

Two different Petri dish bioassays were set up to evaluate potato plant acceptance in non-choice (potato leaf) and dual choice conditions (potato leaf vs horse bean leaf). In each bioassay, leaf contact and feeding activity were studied.

Plant stylet penetration and feeding activity of *A. pisum, M. persicae* and *M. euphorbiae* were investigated on potato plants with the electrical penetration graph (EPG) technique.

The contribution of *Acyrthosiphon pisum* to the transmission and spread of non-persistent phytoviruses is discussed.

MULTIFUNCTIONAL TECHNOLOGICAL SYSTEM FOR BENEFICIAL INSECT MASS REARING

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Summary

The ability to rear insects under controlled conditions has long been regarded as desirable or necessary to facilitate research on many aspects of entomology. It is integrated in the informational system used for assisting the measures of sustainable utilization of the natural fond, as a fundamental layer of viable economical development and social welfare which necessitates a new approach on the biodiversity conservation strategy. Considering the recommendations of the Biological Diversity Commission, this project is focused on defining the populations/species as service rendering units according to the model introduced by Luck et al. (2003). The concept allows the approach of the biodiversity conservation from an innovating perspective, focused on the biodiversity value for the society for a better development of the decisional process. The research activity within this field is constantly developing worldwide, under the influence of numerous local and global factors, as well as the clarification of some basic research aspects. Development of these systems materialized into a series of technical achievements which allowed defining the fields in which alternative means can be integrated in order to control the pest problem in agriculture. Along with the first industrial and experimental successes the resulted products, meaning the useful individuals or insect stages were defined, classified and introduced for sale under the name of "agro-biological products" in the plant protection field, at convenient prices.

In the present paper the main components of the system elaborated will be designed and the work technical and physical parameters established:

- containment system;
- climatic system;
- light control system;
- relative humidity control system

TECHNICAL AND PHYSICAL PARAMETERS MONITORING OF THE MULTIFUNCTIONAL TECHNOLOGICAL SYSTEM

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Summary

This paper presents recent contributions and engineering developments and equipment specifications necessary for constructing a workable, economically feasible rearing multifunctional technological system capable of supplying a production of individuals from beneficial insects populations. This system should also be of value in developing different levels of technology for the mass rearing of other insects, pest insect including.

Engineering developments are discussed in this paper, and specifications necessary for technical and physical parameters of the system monitoring are presented. The construction of the system are versatile, modular concept to provide automated electronically adjustable environments parameters, i.e., accurate temperature, humidity heating and air filtered against airborne contaminants.

The room's climatic parameters are digitally controlled by the system designed in the block diagram of controlled-environment system showing the interfacing with all of the six chambers. The extended-memory unit the SDI-12 M512 interface. The complete system is composed by a central data-control bus links the six chambers in a parallel configuration to the control system. Since the HPxw4400 Workstation employs discrete-component logic and the remainder of the system is built around logic-level converters (wireless sensor interface) which are used to process the incoming and outgoing signals for system compatibility. The basic control scheme consists in four phases of each of the six chambers:

- measuring;
- data transmission;
- data processing;
- remote control unit

During the first phase, the controller (Metrilog T707 unit) sequentially addresses each chamber, requests temperature data from the chamber, and then compares the received information with the desired programmed environment. The controller then transmits the appropriate command signal (temperature on or temperature off, or changing the desired level). Once the first phase has been completed, the second phase is initiated. The second phase is an RH scan and performs essentially the same tasks, turning the humidification circuit on or off. Consecutively the system has initiated the feedback turning on or off digitally all the sensors (relative humidity, MC light RF modem, radio data logger and so on)

TECHNOLOGY OF MASS REARING AND INNUNDATIVE RELEASES OF *PODISUS MACULIVENTRIS* SAY (HETEROPTERA:PENTATOMIDAE) FOR ECOLOGICAL BASED PEST MANAGEMENT (EBPM) IN THE POTATO CROPS FROM ROMANIA

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The possibility of use in the biological control of pests various natural enemies is now a necessity more than ever. The use of entomophagous insects as biocontrol agents implies the availability of adequate rearing techniques enabling large-scale production at low costs. The biological control by augmentation of beneficial arthropods is a strategy to increase the effectiveness of predators or parasitoids by mass production and periodic colonization.

Our researches establish for the first time in Romania that the species Podisus maculiventris Say could be promising agents for the biological control of larvae of L. decemlineata in the field conditions.

P. maculiventris was reared under controlled conditions in the small bio-station at Research Development Institute for Plant Protection - Bucharest. The technical line and ensemble of facilities had been described in the present paper. After the tests of mass rearing, the basic host M. domestica (four instars larvae) is recommended for predatory feeding because of its economically growing in industrial facilities.

The results obtained establish the moment and rate of release into the crop and effectiveness of predatory rate against Colorado potato beetle in four regions of Romania.

MECHANISATION

MECHANIZATION LEVEL AND PROBLEMS OF POTATO GROWING IN TURKEY

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Keywords: potato *(Solanum tuberosum L.),* mechanization level, equipment, Turkey

Abstract

The aim of this study is to determine the mechanization level and potato growing problems come across at practice in Turkey. Farmer point of view, interviews with manufacturer union and manufacturers take part in the paper. One of the most important factors for yield and quality of product is to use advanced technological machines and equipment in agriculture. Generally, equipments used in potato growing for tillage, hoeing, plant protection and irrigation are the same as the other agricultural crop. In Turkey, most of the farmers are using potato planters for hoeing in practice, too. Semi automatic and full automatic planters for planting; plough, tractor mounted potato spinner, shaker digger and self-propelled potato harvesters for harvesting are using in our country. It is very important to use special equipment for planting, harvesting, grading and cleaning in potato growing for getting high yield and good quality crop.

INTRODUCTION

Potatoes are grown in Turkey on an area of approximately 160 thousand ha, with an yearly production amount of 5 million tons. 55% of the production is done in the Central Anatolian and the Pass region, 21% of it is done in the Black Sea and the North Pass Region, 16% in the Aegean, Mediterranean, and the Marmara Regions, 8% in the Eastern Anatolian Region (Arıoglu et al., 2006). Potatoes are grown in the Aegean, Mediterranean, and South East Anatolian regions as an early product, while it is grown in the other regions as the main product. Potatoes are grown more or less in almost every province except a few provinces, where it is not grown due to negative climate conditions. (Arıoglu, 2002).

The use of mechanization equipment in our country changes due to different factors such as the climate conditions, the structure of the land, the inclination of the area, the size of the area, or the economic income levels of the producers (Kara, 2006). For instance, the potatoes producers in Central Anatolia where the area is quite flat benefit from mechanization at optimum level, which many producers in the Black Sea Region where the area inclination is quite high, still use hand hoes for the planting, caring and harvesting procedures (Aytac and Arslanoglu, 2005). In general, the equipments used for cultivating the land, hoeing, filling channels, treating with pesticides, and watering are similar with or the same as the equipments used for the other plants.

This article uses individual interviews with potatoes producers, the producer union, and the producers themselves, and tries to determine the state and problems of mechanization in growing potatoes in Turkey.

Land cultivating equipments: Cultivating the land correctly and in the right time is an important start for a quality and high produce in growing potatoes, as it is also in all the other plants. One of the most important equipments used for cultivating the land is the plough. The producers growing potatoes usually cultivate the land 1 or 2 times with a depth of 35-40 cm before the planting begins. Depending on the land structure and the power of the tractor, ploughs with 2-3-4 blades are used. Another equipment that is used (not so much) is the subsoiler. In production areas with a high amount of clay in the soil, a subsoiler with a single blade is used to plough 60-65cm deep once, and in the subsequent tilling, ploughs are used. The equipment that is used most, for cultivating any kind of land in our country, is the cultivator. Cultivators which are produced by local firms, have suspended types, not suspended types, and types with side suspension.

Potatoes planting machines: Although the use of mechanization in planting potatoes has increased in our country since the 1990s, the producers are doing their planting with hand tools (hoe), winged ploughs, half and full automatic planting machines, depending on their conditions and their mechanization levels (Altuntas and Kara, 2001). More or less human power is needed for all the planting methods except the full automatic machines. Since the planting cannot be done in a homogenous depth, the development of the plants is irregular. 2 workers work at each machine in this type machines, and they can plant on an area of approximately 2ha a day. But for the last five years the firms producing industry potatoes brought full automatic planting machines from abroad, and on an optimally cultivated agricultural area they can plant on an area of 8-10 ha a day, decreasing also the number of the workers. It has been observed that in planting done with this type of machines the plants develop homogenously.

Channel filling and hoeing machines: Chain filling and hoeing are cultivation processes which not only controls the weeding but also has a direct effect on the root and knoll development of the potatoes and the capacity and quality of the knolls. It is known that a hoeing and channel willing of at least 2-3 times has a positive effect on the potatoes yield. There is still no sufficient amount of modern equipment in our country that is used for channel filling. The producers use local equipments such as planting machines and single blade ploughs for this purpose. In small family producers, however, the hoeing and channel filling is done with a hand hoe and with a horse.

Equipments for applying pesticides and for watering: Most of the producers growing potatoes on large areas apply pesticides against weeds at least 1-2 times, while in small producers they are controlled with hoeing. All the producers apply pesticides against the Colorado potato beetle *(Leptinotarsa decemlineata Say.)*. Depending on the size of the producer, backpack sprayers, or sprayers which are powered from the tail spindle of the tractor are used for the applying of pesticides. The watering equipments are the same equipments that are used for the other plants. In general, a mixed method of watering or rain watering methods are used. The producers say that no dropping watering method is used.

Harvesting machines: The harvesting is done with hand hoes, with ploughs, or with half or full automatic machines, depending on the size of the producer, the inclination of the area, the structure of the land. In agricultural areas such as the Black Sea Region, where there is a high inclination and mechanization is difficult to apply, the potatoes are harvested with hoes in small family producers. Special potatoes harvesting ploughs are used for the harvesting with ploughs. In this harvesting method, the harvesting parts which are fitted instead of the blade plough body, go through the middle of the lines and digs out the potatoes under the ground. The knolls are collected by workers by hand and put into sacks. This equipment is used more in the Mediterranean Region. The knolls are damaged during the harvesting, and there are partly harvest losses, and the cost of the workers is high. Half and full automatic harvesting machines; These are half automatic harvesting machines which are driven by the tail spindle of the tractor. In these machines, the knolls are dug out and placed together with the soil still stuck on them onto a moving mechanism with holes, and move there. Meanwhile, the small soil parts drop down from the filters, while the knolls and large soil parts drop to the sides. The potatoes are collected by the workers and put into sacks. The cost of workers increase, and the knolls are partly damaged. The equipments, which are used primarily in the Central Anatolian and the Pass regions and the Aegean Region, where especially potatoes cultivation is great, are produced by local firms. In other half automatic harvesting machines, which use the same system, the knolls that are dug out from the soil are filtered and stored, while workers at both sides of the machine classify them according to their size and put them into packages . In harvesting which is done with this method, the knolls are not damaged, but the harvest loss is quite high. In full automatic harvesting machines the knolls which are dug out from the soil are filtered and then collected in the storage on the machine itself or they are transferred to a trailer which moves together with the machine. The heavy soil parts and plant rests which are transferred together with the knolls are cleaned with various filter systems after the harvesting, but before they are stored. There are good results in plain lands. These machines which have been used (though not so frequently) during the last years are not fitting for clay and heavy soil types.

In our country, the harvested potatoes are stored in sacks in small producers. There is a loss of knolls of 20-25% in these types of storage, and the costs increase. In bigger producers, there are systems which can carry out size classification and storage procedures in heaps in underground or aboveground storages, under controlled conditions. Especially in the Central Anatolian Region where there is a great amount of potatoes growing and where a great part of the grown potatoes are stored, the control of the temperature, moisture, and ventilation of the underground natural stores is done by automatic systems, and equipment for the decrease of the amount of storage losses is used.

RESULT

The potatoes production areas in our country are different from each other in terms of geographical structure, land and climate features. This limits the use of mechanization. According to the general result which was obtained after talking to the producers, the producer unions, and individual interviews done with the producers, it has been seen that the use of mechanization for small family producers is not economic, when we consider the sizes of the producers, and the economic income levels of the producers. Increasing the use of appropriate equipment in bigger producers, taking the land structure into consideration, and making use of equipments which have been produced by local firms, and of new technological developments, and developing these according to the structure of the land and the region will decrease the cost of workers and the loss of the products and therefore increase the economic gain.

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“BARBUTE”: A NEW ECHIPAMENT TO LIMIT STREAMING POTATO CROPS

« Barbutte » : A new equipment to limit streaming in potato crops

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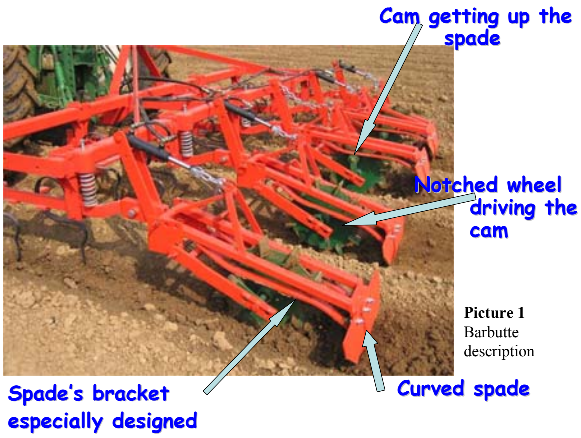
Abstract:
Due to its deep and fine soil preparation and the specific design of the plantation, the potato crop is very sensitive to streaming during at least the 2 months following plantation. Designed by Dormy Ets and now distributed by Cottard Company, the “Barbutte” equipment creates mini dykes regularly spaced between the rows. Practical experimentations carried by several technical groups managed with ARVALIS-Institut du végétal in northern France showed the efficacy of this simple tool for eliminating mainly the risk of streaming and its collateral dangers (erosion, pesticides transfer…). Measurements made at harvest time showed no effect of this technique on yield and tubers quality.

Key words: Barbutte, Erosion, Mini dykes, Streaming

Introduction:
In order to create sufficiently important ridges of fine soil for a good development of tubers, soil preparation for potato crop is usually deep and done with a strong fragmentation of aggregates. Considering also the specific design of the ridges, this creates a high risk of streaming and collateral problems (erosion, pesticide transfer). Searching more secure cultivation on these aspects, Dormy Ets imagined a special and simple equipment creating mini dykes regularly spaced between the ridges. This equipment is now distributed by the Cottard Company [1].



Picture 1
Barbutte description

Barbutte equipment:
The equipment used curved spades working between the rows regularly and mechanically got up by cams driven by notched wheels (pic.1). It can be used alone or can be added directly on the planter or the ridger. On the planter the movement of the spades is directly taken on its wheels. The created mini dykes are regularly 1,70 m spaced and initially 10 to 12 cm high. Their base is quite large (minimum 30 cm) and give them a good stability even during heavy rains (pic. 2).

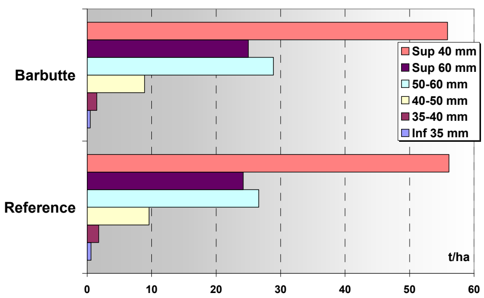
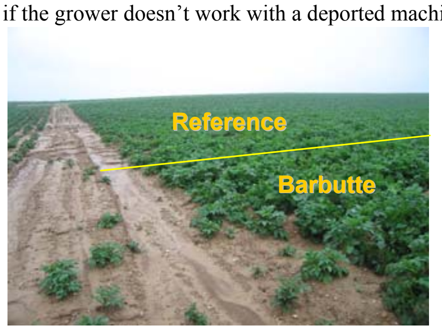


Figure 1
Effect of Barbutte on crop productivity (Average of 2 trials)

Picture 2
Barbutte working in a sloped field in Champagne region

Fields trials:
A survey carried in the northern France by different technical groups (AREAS, ARPTHN, ATPPDA, CAT 51, CA59/62, Ceta de Ham) with the collaboration of ARVALIS – Institut du végétal in several fields during the 2 years 2006 et 2007 showed a very good comportment of this equipment in loamy clay soil to avoid any streaming even during heavy rainy events (pic. 3). The good stability observed for the mini dykes during the whole vegetation period obliges to use a complementary specific tool to destroy them at last at the harvesting time if the grower doesn’t work with a deported machine.



Picture 3:
No streaming and no erosion traces after an heavy rain of 60 mm in 3 hours (Béthencourt – night 7/ 8 June 2007)

The measurements done in the different fields didn’t show any significant differences between the field‘s part where Barbutte equipment was used and the reference, as for productivity than for tubers quality (fig 1-2)

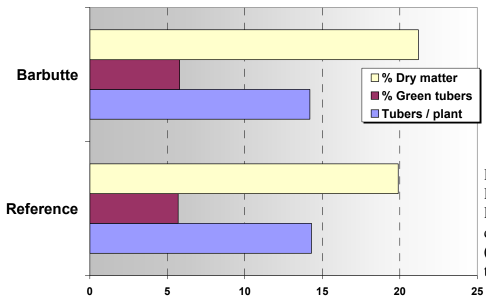


Figure 2
Effect of Barbutte on crop quality (Average of 2 trials)

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TESTING METHOD TO ASSES THE POTATO TUBERS BEHAVIOR TO MECHANICAL IMPACT LOADINGS

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INTRODUCTION

Economic losses due to potato bruising are significant. The potato industry needed to know not only how to improve harvesting and handling equipment and operations, but how far tubers could be dropped safely in handling equipment and under what conditions. Impact damage to potato tubers appears as a result of impacts and compression. Substantial damage in potatoes occurs during harvesting and handling operations. The impacts occur primarily when the tubers strike hard surfaces or each other while being conveyed, or in dropping from one conveyor to another. Current technique allows the utilization of some methods that will reduce mechanical impacts by reproducing in the laboratory the process which harm the tubers under mechanical loadings produced by external forces [1]. The pendulum technique provides the assessment of impact accelerations precisely and reproducibly. This paper presents a testing method of studying the potatoes to impact damage under short time mechanical loadings. The three types of potatoes: Roclas, Nicoleta and Dacia were tested in the laboratory conditions by means of a computer controlled pendulum.

MATERIAL AND METHODS

The potatoes from three varieties *Roclas*, *Nicoleta* and *Dacia* were planted in April and harvested in October . Two levels of potassium were given to each variety in order to induce differences in susceptibility to tissue discoloration. The potatoes were harvested manually and stored individually on soft ventilation pads to minimize mechanical damage. The first experiments were done in January, three months after harvesting [3].

A number of 90 potato samples from each of the Roclas, Nicoleta and Dacia varieties were chosen for experiments. Next the potato samples from each variety were divided in three groups of 30 potatoes. Each of the three groups of potatoes had different fertilization level notated with: V1, V2 and V3.

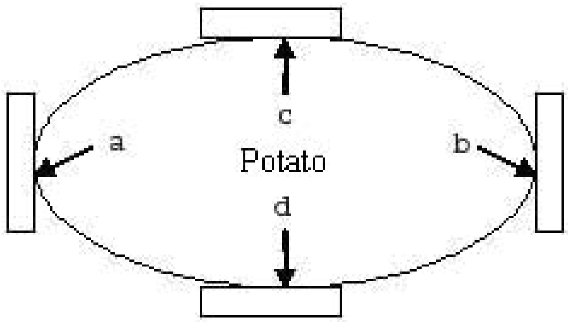

Fig.1. The impact positions

The experiment begins with a group of 30 potato samples from a variety. Every potato sample is separated, marked, fixed in the pendulum holding system and impacted in four different positions (a,b,c,d) situated on the potato length and wide extremity. In order to generate the impacts a computer controlled pendulum is used and its scheme is presented in the figure 2. The pendulum arm *4* is a cylindrical metal tube with a length of 600 mm fixed on the top at the mainframe 1 directly on a rotary encoder ax *7*, which registers the rotation angle of the pendulum arm. The acceleration after impact and deceleration during the impact are measured by an accelerometer *2*, fixed on the arm of the pendulum, at the backside of a non-spherical impact body *3* .

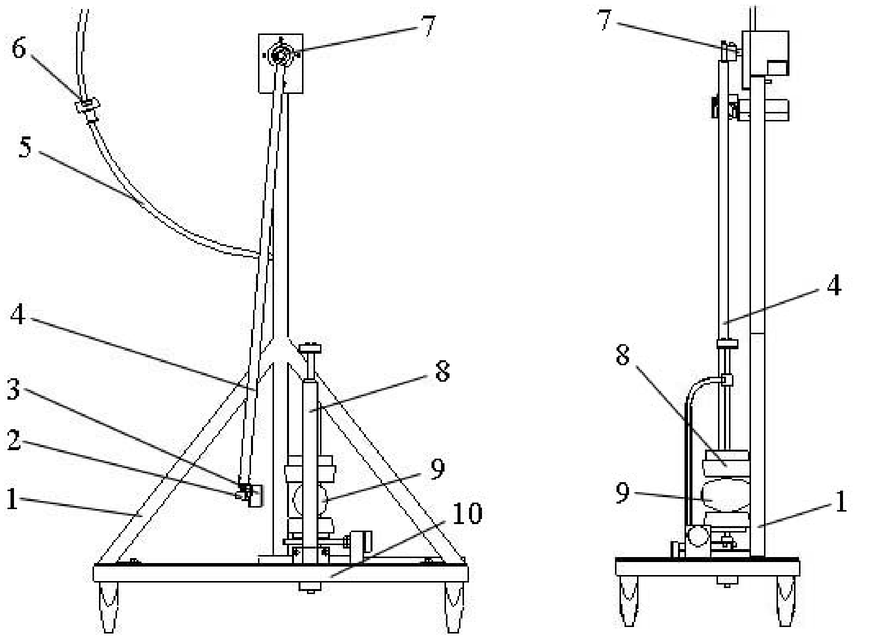

Fig. 2 The schematic representation on pendulum [2]

An electromagnetic system *6* mounted on the circular support *5* fixes the pendulum arm in a position necessary to administer the desired potential impact energy. The electrical signals produced by the accelerometer and encoder when the pendulum arm is released by the electromagnetic system, are isolated and amplified by a data acquisition system.

Another element of the pendulum is the sample holding system *8* , designed to fix the potato *9* and to avoid supplementary loadings.

Testing the samples by means of computer controlled pendulum has several phases. First the sample is fixed in the potato holding system and guided until the sample impact point and impact body position are identical and the angle of the pendulum arm is 0. This procedure is presented in figure 3.

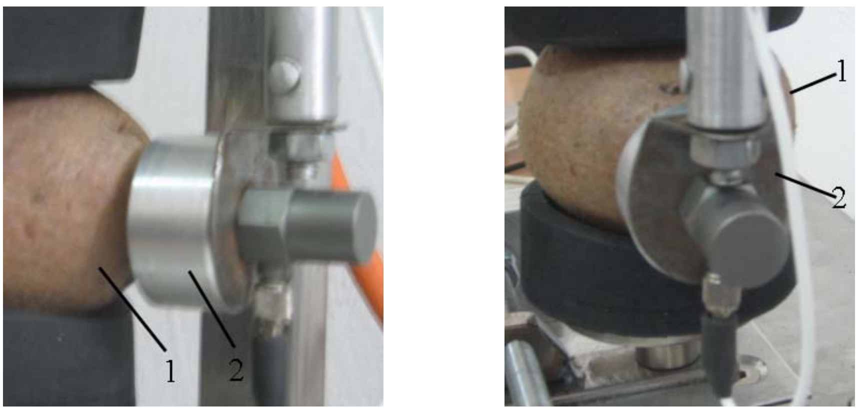

Fig 3. Settling the impact point 1 – potato ; 2 – impact body

For this position the potato guiding system is blocked and the system is calibrated with a Labview application created and used for data acquisition and processing. The pendulum calibration pannel is presented in figure 4.

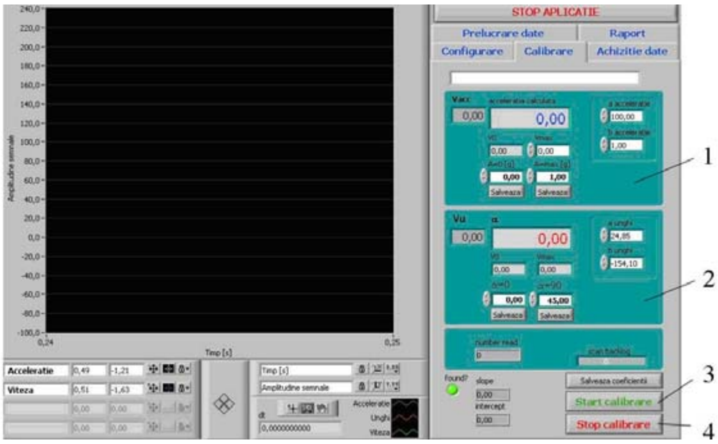

Fig. 4. The pendulum calibration using a Labview application 1- area for accelerometer settings; 2- area for pendulum arm calibration; 3 – button to start the calibration; 4 - button to stop the calibration

The pendulum arm is lifted manually to the required position angle necessary to deliver the initial energy E_i where is arrested by an electromagnetic device presented in figure 5. By a computer command the pendulum arm is released to free-fall and hits the potato samples.

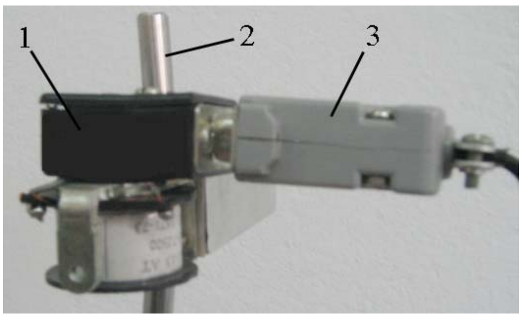

Fig. 5. The electromagnetic device 1- spool coupling, 2 – holder, 3- electrical connection

RESULTS AND DISCUSSION

Following the procedure presented potato tubers from the classes Roclas, Dacia and Nicoleta were tested after 160 and 320 storage days. The aim of testing was to determine if the storage period influences the potato elasticity.

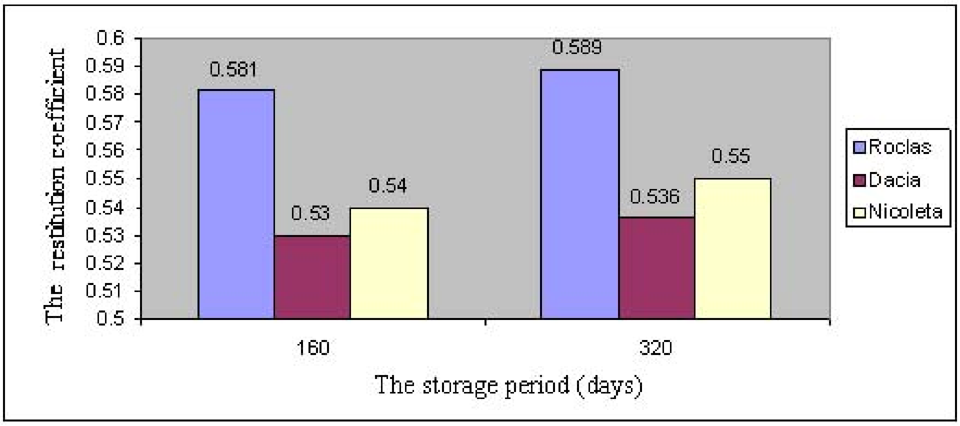

Fig.6. The influence of the storage period in potato elasticity

Using the computer controlled pendulum the impact restitution coefficient was calculated from the pendulum arm velocities before and after the impact. The results for an initial impact energy of 0,072 J are presented in the figure 6.

The graphic showes that the storage period determines the increasing of the restitution coefficient which leads to a higher elasticity for all the three varieties. Also the highest elasticity was discovered for the tubers from Roclas varieties followed by Nicoleta and Dacia.

CONCLUSIONS AND FUTURE WORK

During experiments, the computer controlled pendulum proved to be a very useful tool to impact tubers to short time mechanical loadings precisely and reproducibly. The pendulum provides the assessment of delivery and absorbent energy, measurements of approach and rebound velocity and acceleration, accurate and repeatable drop height control. A Labview program created for the pendulum application allows graphical visualization of the dependences between acceleration and time in the moment of the impact, the number of impacts for a free fall of the pendulum arm as well as the computation of the absorbed energy

The computer controlled pendulum method offers the possibility to study the effect of a wide range of impact energy, impact velocity, impact body shape, time intervals between consecutive impacts and their interactions with the bruise susceptibility [4].

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AGROTRONICS IN MEASURING AND MAPPING THE PARAMETERS OF THE SOIL

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Introduction

Precision agriculture has started as a necessity to decrease inputs in agriculture under the pressure of economic, legislative, environmental and agricultural systems of control and entered as a new methodology that could be the key to solve many current problems. Agriculture precision approach involves systemic factors: biological, ecological and socio-political elements and has the characteristic elements of space and time.

Methodological, precision agriculture incorporates all other methods of research and interpretation of experimental results, based on observations, experiments, analysis and spatial statistics, systemic approach, modeling and simulation process until the use of space technologies peak.

Characterization variability of agricultural land and its consequences on reductions in production and efficient use of resources are possible in precision agriculture due to technical achievements in raising plants, production monitoring, Geographic Information Systems-GIS, Global Positioning Systems - GPS and remote.

Our main goal was the achievement of complex installations for modeling, design and implementation of an intelligent measurement system, acquisition and interpretation of the results for the real-time characterization of the agroproductivs parameters of the soil.

In the field of soil chemistry have studied a series of phenomena and properties such as absorption and exchange cations, various constituents solubility of the soil content and composition of organic matter, the content of Fe, Mn, Al oxides, content and variability of micro-nutrients.

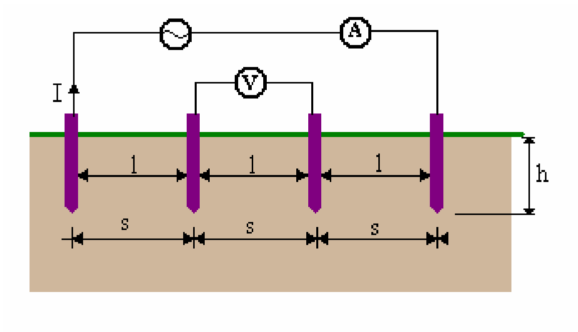
Physical, and in particular hidrophisic properties of the soil and how they are influenced by agricultural technologies have made the subject of study of soil physics. Outstanding results were obtained in mathematical simulation of ground water and balance role in the formation of the harvest.

Experiences have been located on land NIRDPSB Brasov on the ground cernoziomoid cambic, characteristic of about 5% of the agricultural land in the county and about 15% of Barsa depression .

A soil property which may affect the variability of the harvest is materialized in a series of maps which will lead the implementation of precision agriculture, the maintenance of environmental quality. Usually, the maps on soil properties are obtained by taking samples of the soil, followed by the analysis procedure. The data become interpolate levels in the prescription quantities of fertilizers, herbicides and substances, but the representation of soil properties is limited to the costs involved by the increased number of the samples required. For the purposes of the improvement of these aspects there have developed a series of automated equipment with soil sensors used to generate a DGPS certain maps private properties of soil. So there are, in the prototype stage or even commercial, a series of portable sensors that allow data collection during displacement of the equipment: electric and electromagnetic sensors, optic and radiometric sensors, acoustic sensors, pneumatic sensors, mechanic sensors, electrochemical sensors, each highlighting having regard to other properties of the soil.

The four terminals method of measuring the electrical conductivity of the soil

Electric and electromagnetic sensors use the electric circuits to measure the capacity of the soil particles to lead or to store electric power. When these sensors are used, the land becomes an electromagnetic circuit and changing local conditions affect the signal immediately registered in a database. The method of measuring the electrical conductivity of soil using contact sensors involves the construction of a device having two or three pairs of electrodes-knife. A pair of electrodes is used to apply a ground power, while the other two are used to measure the fall of voltage, electrodes are set to measure the conductivity apparent either by a superficial reading, at a depth of about 0- 30 cm, either by reading a deep, a depth of 0- 90 cm (Figure 1). It was designed and carried out an experimental model to measure the electrical conductivity of the soil using the method with four terminals.



h = depth buried electrodes
V = tension between the bars in the middle
I = current external input with bars

Fig.1 The four terminals method of measuring the electrical conductivity of the soil

The conductivity measurements compared with references geological using an external GPS receiver and stores the results in the form of digital. Data registers on a range of 1s, the density of data can be modified by the operator by changing the speed of travel and/or distance between cross-section measurements.

The prototype for measuring the electrical conductivity of the soil

The portable sensor (fig.1) shows up the components: the external electrodes 1 and 4, the internal electrodes 2 and 3, the screw nuts 5 for electrodes fixing on support, the washers of safeties 6, the rings of connection 7, the conductors of electrical current 8 and 9, the elbow from plastic which assure the ergonomics of the sensor 10, the vertical and horizontal plastic tube 11, the connectors for the liaison electrodes to the source of current 12.

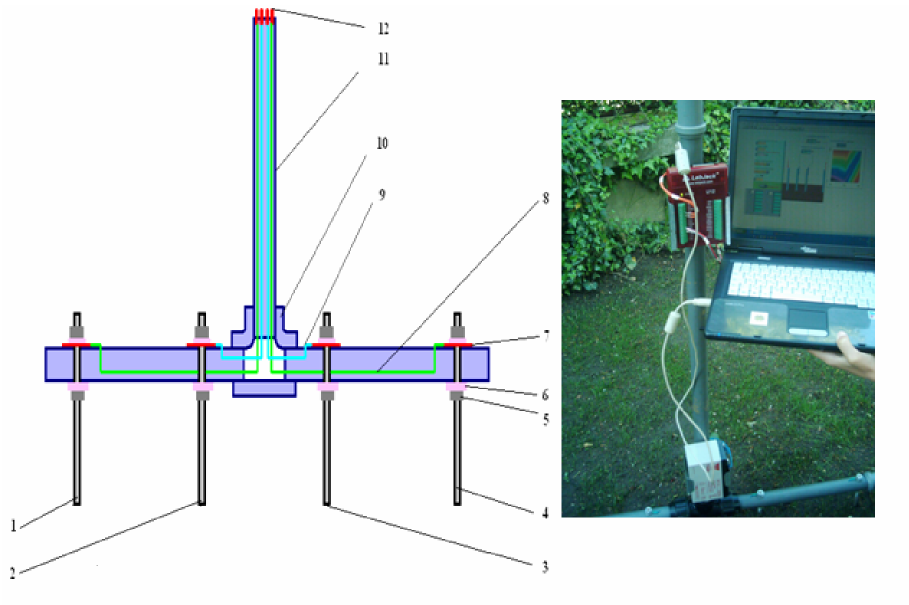


Fig. 2 The prototype for measuring the electrical conductivity of the soil

The virtual instrument for mapping the electric conductivity of the soil

Project execution on the software used involved building a virtual instrument for displaying the values of electrical conductivity of the soil. For this purpose we used graphics software programming LabVIEW, version 7.0., a product that National Instruments offers a unique environment for development through the system or graphics programming. It brings solutions for advanced scientific applications, industrial process automation, communications inter-network applications and the use of dynamic libraries in Windows (DLL), query databases (SQL), the management and regulation of industrial processes.

The basic concept of the program is that LabVIEW virtual instrument, a graphic representation which resembles the largest possible extent with a real physical instrument. The application developed under LabVIEW provides to the user a panel that can handle a series of virtual instruments. Labjack is a counting and automation device using USB (fig.3); it provides analog and digital inputs and outputs. Data Acquisition Device offers an easy to use interface between computer and physical world, reading out the sensor that measures: tensions, current, power, temperature, humidity, wind speed, force, pressure, mechanical tension, acceleration, rotations, light intensity, gas concentrations.

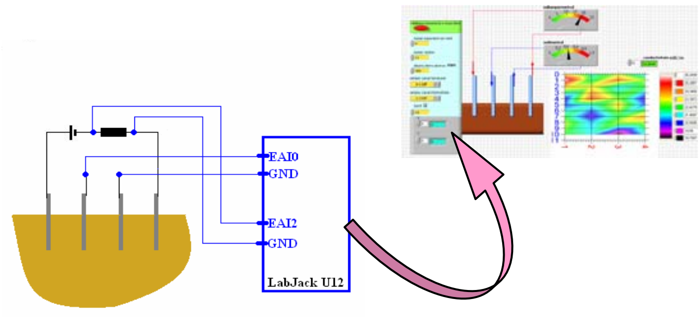


Fig. 3 Connecting sensor and the data acquisition device.

On the panel the user can set the signal applied to the two electrodes and will read the column displayed in the corresponding voltage electrodes 2 and 3 and current from the electrodes 1 and 4. The program has provided a button to validate the following reads, so this is carried out at the request of the user (fig.4).

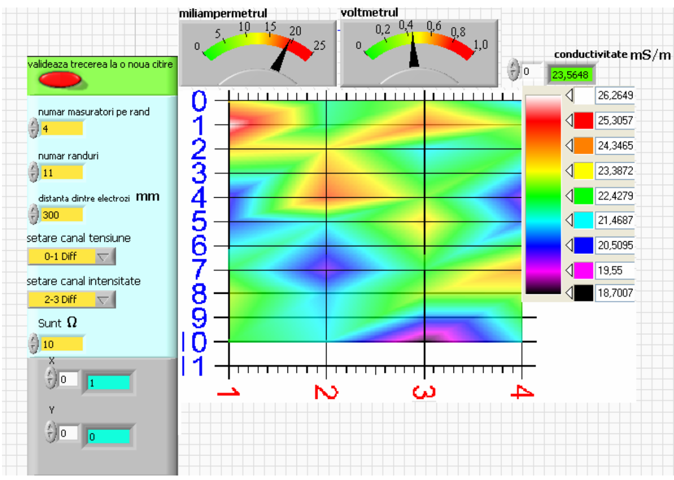


Fig.4 The panel of the virtual instrument for mapping the electric conductivity of the soil

In the diagram there is a repetitive structure For that allows the execution of a number of measurement equal to the constant connected to the terminal N of the loop, and the entry of data into vectors corresponding to electrodes 1 and 2 for the voltage and to electrodes 3 and 4 for the current. In the structure is located the EAnalogOutput.vi function which provides voltage levels desired by the user. Also are located and EanalogInput.vi two functions that allow the second reading signals in voltage. Initially provided arrays, with values void, were hidden to the user, they having role in creating the physical storage space values read from sensors. The elements of these arrays are replaced by elements whose new element pin is connected to the two EanalogInput.vi functions. The chart also contains functions for calculating the electrical conductivity of soil, and the transformations necessary for displaying the outcome of final mS/ m .

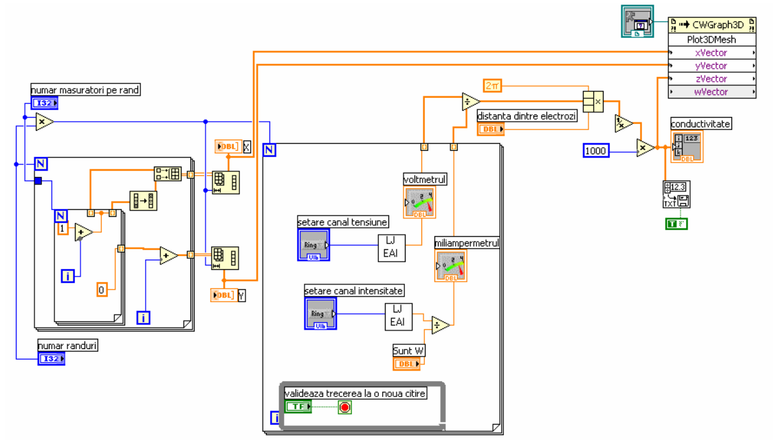


Fig.4 The diagram of the virtual instrument

Conclusions

The plotting technique of geological sub-layers by inducing electric currents in the ground and by noting the distribution of the potential field resulted have been develop at the beginning of XX-th century by Conrad Slumberger. Later on, the measurement of soil electrical resistivity was applied in a multitude of areas: determining the rocks lithology, plotting the aggregates and clay deposits, plotting the subterranean water, locating the geo-thermal areas, plotting the archaeological sites.

This the graphic instrument allows, through the connecting-up to a mobile system of sensors, the gathering of the date of the electric conductivity of soil in much more less time than in variant width a human operator gathering proofs of soils. The automatic generating of electric conductivity of the soil maps, for large surfaces permit the decrease number of collecting points of the proofs of soils, just some of them from the zones of different conductivities.

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CONTRIBUTIONS OF THE PRECISION MECHANICS AND MECHATRONICS DEPARTMENT OF TRANSILVANIA UNIVERSITY OF BRASOV AND OF THE NATIONAL INSTITUTE OF RESEARCH AND DEVELOPMENT FOR POTATOES AND SUGAR BEET BRASOV IN PRECISION AGRICULTURE.

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Introduction

The entire world faces three major issues generating different problems and conflicts, more or less generalize: the demographic increase, the restraint of the food resources, the pollution and the deterioration of the environment. Agriculture can and must interfere in solving this major problems. The agriculture of the past years, the performances realized in the developed countries, the technologies used, outline the agriculture of the third millennium.

The main objective of the modern agriculture is represented by the continuous improvement of the production process. This is possible only by modernizing the concepts and the research methods in the agricultural practice, by using the recent scientific achievements, the experimental and the calculation techniques, as well as the interpretation of the results.

The precision agriculture repents a new method that may represent the key for solving many of the up-to-date problems.

The opportunities for a precision agriculture are:

- The capacity for comprehending the complexity of the agricultural systems – systemic and holistic approach;
- The capacity to monitories the phenomenon and processes – the automatization of the data acquisition;
- The achievements in the computing techniques – hardware, software, fireware and data bases;
- The improvement of the computing and interpretation methods – statistics, modeling simulation, decision supporting systems – DSS;
- The development of the geographical informational system – GIS;
- The appearance and development of the spatial analysis and statistics – teledetection, GPS;
- Technical achievements in the improvement an automatization of the machines used in agriculture - mechatronics in agriculture.

Mechatronics in agriculture, as an important part of the precision agriculture, represents the entire intelligent Devices and Implements – IDI, which represent part of the machines used in agriculture and of the research equipments and which enables the monitorisation of the production conditions and the control of the inputs. The main components are the computing technique, the GPS, electronic hydraulic and mechanic equipments. A special type of IDI is represented by the Variable Rae Technology – VRT, which allows the application of the chemical manures and pesticides in different rates, depending on the requirements settled by considering the existing reserve. The differential application is made by controlling equipments (computer and PS) capable to transmit the information from an application map, into commands for the application devices, depending on the variability of the field requirements.

Achievements within the mechatronics domain in connection to the agriculture

The Precision Mechanics and Mechatronics Department with Transilvania University of Brasov and National Institute of Research and Development for Potato and Sugar Beet Brasov have many joined concerning and achievements with respect of the use of mechatronis principles and devices in agriculture, such as:

A. **Computer aided automatically installation for measuring the content of starch and dry substance from the potato's tubers**

The measuring installation is based o the determination method of the dry substance and starch from the potatoes. This method consists in their double weighing in air and water. This way, the test specifically weight is calculated. Between it and the content of dry substance and starch, there is a linear dependency, which is described by the equations with coefficients given some elsewhere in the literature.

The installation has the next parts (see fig.1)

- The electronic computer (1)- allows the command, the control and the calculation of the values appropriate to the content of dry substance, starch, proteins and saccharine, that were reduced from the potato;
- The commanding electro-mechanically device, (2) –assures the displacement by means of a continuous current motor-reducer and a mechanical transmission for the water reservoir (3) with the aim of potato immersion(4);
- The electric system (5) – commands and controls in PWM regime of the continuous current motor-reducer, which trains the action electromechanical device;
- The support (6) realized from an aluminum alloy for sustaining te electronic balance and the basket with samples;
- The electric balance of SARTORIUS IQ 4100 type (7) measuring with a 0,03% accuracy and a 0,1g resolution, and which is equipped with a srial interface for transferring the measured data and also for transferring the commands between it and the computer;
- The printer – assures the printing of the results.

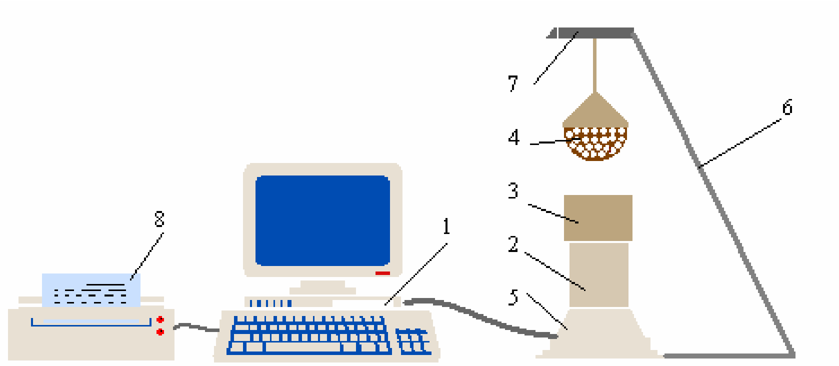

Fig. 1 Computer aided automatically installation for measuring the content of starch and dry substance from the potato's tubers.

The installation was studied, designed an constructed at The Precision Mechanics and Mechatronics Department and is used in research, for the improvement of the potato's tubers and in production, for the correct reception of potato, according to their value give by the content of starch and dry substance from the potato's tubers.

B. Installation for the automatically dozing of the chemical liquids used as manure

The installation for the automatically dozing of the chemical liquid may be use in any application where the getting of different mixtures from each liquid is wanted. Each dozen allows the releasing of a fix volume of liquid (50 cm^3), in the end obtaining a mixture in which every liquid is a module of this quantity.

The installation was designed, implemented and tested by the researchers in the biochemistry of plants in vegetation vessels. For these researchers there were introduced in the vessels mixtures of different chemical liquids so that the evolution of the plants could be observed and also so that, tis way, optimal solution for each type of plant and each climate conditions should be found.

The installation should provide:

• The substantial increase of the work productivity (to allow a working speed of 2000 vegetation vessels a day);
• The resistance of the materials which are in direct contact with the chemical liquids;
• Work precision;
• Reliability;
• Automatisation;
• To allow the storage and o attach to the sample number of the quantity of the chemical liquids dozed.

C. The research, the testing and the devices of a mobile lab as well as the acquisition and automatically processing of the phytoclimate data from the potato crop

Taking into consideration the development in the agricultural domain where far more complex devices must function directly in the experimental field in various weather and climate conditions, it was raised the problem of sing mobile labs with much bigger displacement velocity among the different working points, and which have optimal functioning conditions for the devices and for the research stuff (controlled climate, water and electric energy alimentation source, etc.).

The mobile labs was equipped for a complex data acquisition system for the phytoclimate (15 parameters among which are: the temperatures in different points, the atmospheric humidity, the sun shining interval, the wind seed, the direction of the air current etc.). these date ar processed automatically in order to be used in the agricol research.

The assembly of he main subassemblies in the interior of the mobile la was realized this way (see fig.2):
• The access door (1);
• The windows from smoky Plexiglas (2, 3, 4, 5);
• The washer (6)- used for washing the sample sand for other activities necessary in te mobile lab) supplied by a water store , it's starting being given by an electric pump (the pump functions with the energy from a 12V battery or alternative current of 220V);
• The fridge (7) supplied y a 12V battery or alternative current of 220V);
• The closet (8) in which is put the numerical computer, the printer and the other interfaces;
• The balance (9) used in the measuring of the plants perspiration;
• The working space (10) for the other necessary devices in research concerning the physiognomy of the potato;
• The working space (11) necessary for placing the transducers and other accessories;
• Rotary chair (12)

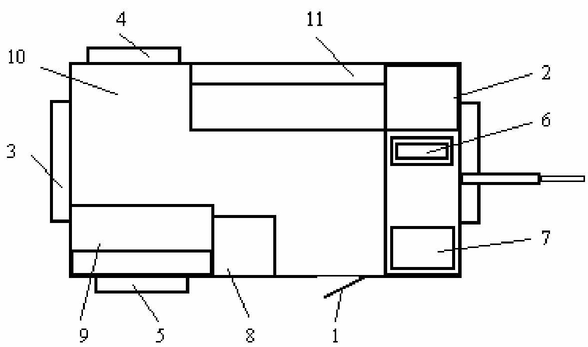

Fig. 2 The research, the testing and the devices of a mobile lab as well as the acquisition and automatically processing of the phytoclimate data from the potato crop.

The devices location within the mobile lab took into account ergonomic principles as well as easy access to the electric suppliers and conditions for a good ventilation of working space during the research activities.

D. **Perspectives within the mechatronical domains in connection to the agriculture – soil electrical conductivity measurement with the aim of mapping by the aid of global positioning systems, data acquisition and processing**

The researches carried out in the area of precision agriculture demonstrated the connection among soil's chemical and physical properties and its electrical conductivity. The electrical conductivity of soil is practical produced in the water layer that fills the pits among the particles of soils, and ca be influenced by a proprietary series ale of the soil porosity, water content, salinity level, cations exchange capacity and temperature.

There have been develop two methods for measuring electrical conductivity of soil, one without contact, using electromagnetic sensors, and another, with contact between electrodes and soil. The direct method has as principle of measuring the four terminals method that consists in injecting a current with known value through two external terminals and measuring the voltage between the inner terminals. Taking into account the mechanical parameters, h the depth of terminals in the soil, and l the distance between electrodes we can compute the soil electrical conductivity.

The block scheme for drawing the maps of electrical conductivity of the soil, by using the global positioning, data acquisition and processing systems is given in figure 3

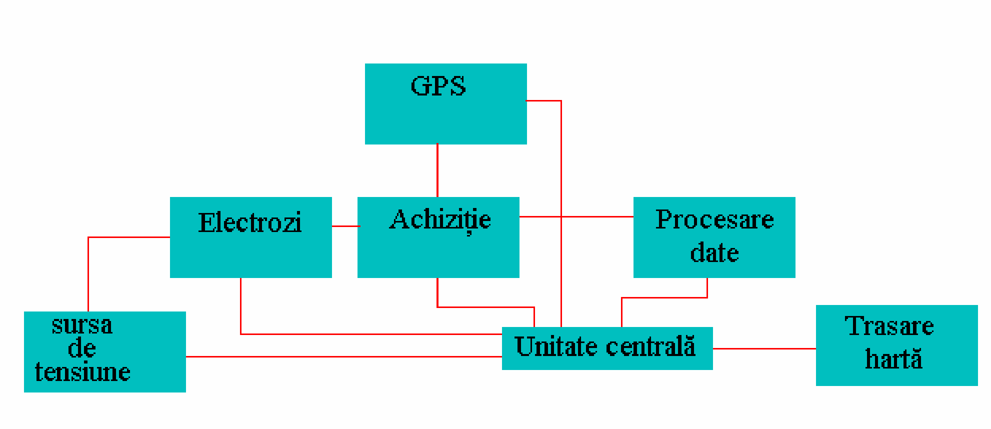

Fig. 3 The block scheme for drawing the maps of electrical conductivity

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STORAGE

STORAGE SUITABILITY OF STARCH POTATOES

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Keywords: starch varieties, storage temperature, storage losses, storage suitability

Summary

In the years 2006 and 2007 eleven starch varieties from the mid early and mid late maturity group were grown on a sandy soil. The temperature regime during storage differed from consistently 4 °C and 7 °C to a triple change between 4 °C and 7 °C. The storages losses between the three temperature regimes showed no clear differences, but with the increasing storage temperature an increase of sprouting and also an increase of the portion of soft rotten tubers appeared. The single varieties react very differently to the three temperature regimes and can be divided on account of these results into three classes of storage suitability.

Introduction

The economic efficiency of the starch potato cultivation is influenced by the tuber yield as well as the losses during the storage. In this process the storage losses gain by the longer processing campaign and the increasing storage duration on farm in importance. For the storage of starch potatoes special buildings with ambient air ventilation are used beside simple storage possibilities in existing buildings and field clamps. The current starch varieties show a high yield and a broad resistance against diseases and pests. Nevertheless the knowledge about their storage behaviour, especially under varying or warmer storage conditions is not up to now enough.

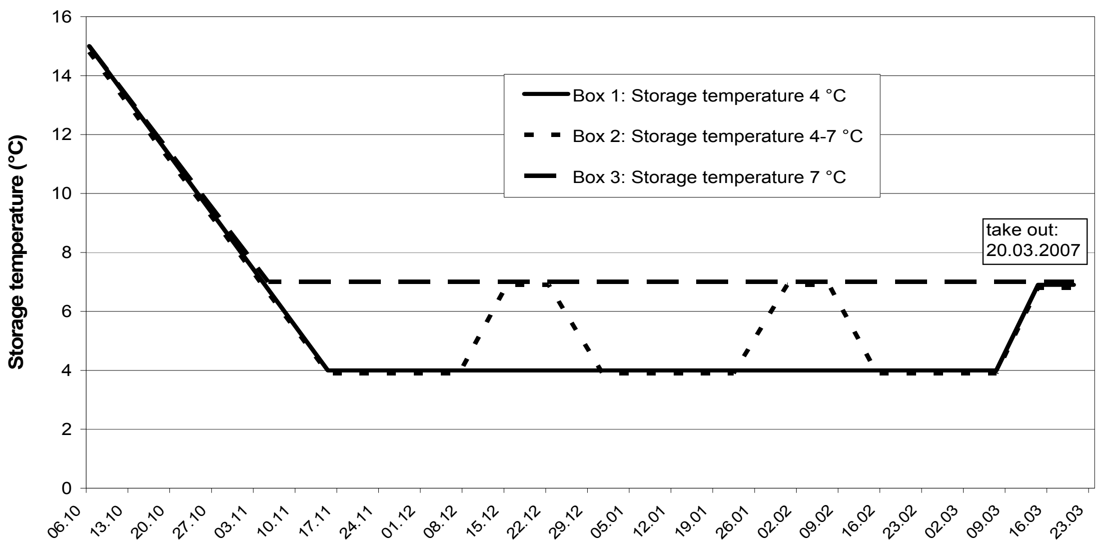

Fig. 1: Temperature curves during the storage season 2006/07

Materials and methods

In the years 2006 and 2007 eleven starch varieties from the mid early and mid late maturity group which distinguished themselves especially by a high resistance to nematodes and potato wart disease were grown on a light, sandy soil with the possibility of irrigation. In the course of the maturing period, the potato vine of the lots was reduced on a mechanical as well as chemical basis. After four weeks the tubers with a good skin set were harvested in each case at the beginning of

October and were stored in the research storage in Dethlingen. In three separate storage rooms with a letterbox ventilation system a temperature regime from consistently 4 °C and 7 °C as well as a triple change between 4 °C and 7 °C was guaranteed with a mechanical cooling system (Fig. 1).

After nearly six months the potatoes were removed in each case in the last March decade and the weight losses, the sprouting as well as the portion of decayed tubers were determined. At the same time the starch content was measured by the underwater weight. In 2007 the susceptibility to damage at harvest of eleven varieties was determined. The tubers of three locations in Germany were mechanically loaded on a web windrower as testing implement. After storing those for six weeks at 10 °C the tubers were peeled and the number of necrotic damages per 100 tubers was recorded.

Results and discussion

On average of the varieties the comparison of the storages losses between the three temperature regimes showed no clear differences (Tab. 1). However, there appeared with an increasing storage temperature a clear increase of the sprouting from 0.3 to 2.8 weight percent (2006) and also an increase of the portion of soft rotten tubers up to 0.4 weight percent (2006).

Table 1: Storage losses of eleven starch varieties (2006/07)

Variety	Storage losses (%)		
	4 °C	4-7 °C	7 °C
1	6.6	6.4	5.9
2	10.0	6.7	6.7
3	7.5	6.3	7.7
4	5.5	5.4	5.7
5	7.8	7.8	9.1
6	6.9	6.4	8.8
7	8.0	6.5	6.7
8	7.1	7.5	9.4
9	5.9	6.4	9.5
10	10.0	13.4	13.3
11	7.1	7.2	7.7
Average of the varieties	**7.5**	**7.3**	**8.2**

The single varieties react very differently to the three temperature regimes (Tab. 1) and can be divided on account of these results into three classes. The varieties whose storage losses stay the same with all temperature regimes relatively (e.g., variety 4) dispose of a good storage suitability for all storage forms. A part of the varieties is suited especially for higher storage temperatures (e.g., variety 7), because under these conditions clearly lower decreases in weight and less sprouting were to be observed. The varieties with a low dormancy (e.g., variety 10) must be stored very consistently at a temperature of 4 °C to hold the sprouting as an essential cause of loss to a certain extent. These varieties are suited neither for a field clamp nor a simple storage in existing buildings.

The investigation of the damage susceptibility at harvest showed clear differences specific to the varieties with a range of about 200 to just 1.000 damages per 100 tubers in the absolute values. A classification of the varieties on average of three locations led to a widely comparable order of rank.

The starch content after storage differed on average of the varieties by comparison of the three temperature regimes also only slightly. But the single varieties react very differently to the three temperature regimes from a decline about relatively constant values up to an increase of the starch content.

CARBOHYDRATE METABOLISM OF STARCH POTATO VARIETIES IN OUTDOOR STORAGE

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INTRODUCTION

In Finland the growing season is short. Almost all starch potato yields are lifted up in September at the latest. Potato starch production factories normally stop running in November and after the latest harvest there is a need for starch potato storage for a couple of months. Storing in an outdoor potato pit is mainly used for starch potatoes. Potato starch production factories have noticed that the loss of starch in the starch separation process is at it´s highest at the end of the factories´ running season and after cold seasons. October and November are often cold, and night frosts are common. It is a known fact that if potatoes are stored at low temperatures, their starch degrades into sugars (Sowokinos 2001). The potato starch industry measures the starch content of potatoes by using specific gravity. Specific gravity measurement can not separate sugars and starch from each other. So potatoes with a high sugar content and low starch content appear as if their starch content is at a good level. Potatoes respond to low temperatures according to the characteristics of their variety. (Barichello et al. 1990).

In this study we tested how the starch varieties that are commonly cultivated by Finnish farmers behave in pit storages where the conditions alternate. We also tested how the cultivars react to storing in cold conditions as compared to storage in steady conditions. The results indicate which varieties are inclined to break up starch into sugars in the different storage conditions.

MATERIAL AND METHODS

Six potato cultivars (Saturna, Kardal, Kuras, Tomppa, Posmo and Seresta) suited for starch manufacturing, were grown at Lammi from May to September, 2004 according to conventional agronomic management. Saturna and Tomppa are early varieties, and their starch content is on an average 17 – 19 % (Kangas et al. 2004). Posmo and Seresta are moderately late varieties with a high starch content. Kardal and Kuras are very late varieties with a high yield potential. Because of the lateness of Kardal and Kuras, growth of these varieties ends usually when night temperatures drop to frost. (Kangas et al. 2004). After lift the yields of all varieties were divided into four lots and subjected to three distinct storage regimes. The lots were: **A)** no treatment, sample analyzed immediately after lift. **B)** 1 week at + 0 °C and 2 days at + 8 °C, 80 % RH. **C)** 6 weeks at + 8 °C, 95 % RH. **D)** 6 weeks in outdoor pit storage, 100 % RH. The treatments consisted of three replicates. The regimes indicated how the varieties reacted to different storage conditions.

The sides of the storage pits were covered by straw bales and the potatoes were covered with plastic sheeting. The measurements of the pits were 3 m wide x 18 m long x 1,5 m high. Information about the conditions in the storage pits (temperature and relative humidity) was collected using HOBO Pro RH/Temp Data Logger.

After treatment the starch content and sugar content of the samples were analyzed. Starch content was analyzed by two methods: by using specific gravity based on the weight of potatoes weighed under water. The underwater weight was converted to a starch percentage according to the EC Regulation No 1949/95. The other used method was the Megazyme total starch assay kit, Megazyme International Ireland Ltd. ICC Standard method No. 168. The Megazyme method measures only the genuine starch content. The sugar content was determined by using The Boehringen-Mannheimin test kit: UV method for the determination of sucrose, D-glucose and D-fructose in foodstuffs and other materials Cat. No. 716 260.

RESULTS

Starch, determined by Megazyme

The starch contents of the samples were at a normal level, when determined immediately after lift (Fig. 1). The cultivars showed a different behavior in starch degeneration in different storage conditions. None of the tested varieties could stand storage in cold conditions. The starch content of all tested varieties went down (Fig. 1). Saturna´s, Kardal´s and Posmo's true, chemically determined starch content was on an average 10 % lower after cold storage than it was on lift. Tomppa´s and Seresta´s starch contents were on an average 15 % lower. Kuras managed best in the cold. It´s starch content was 5 % lower after cold storage than it was after lift.

The optimal storage temperature for potatoes is said to be + 8-10 °C. Within these temperatures starch breakdown should be at it's smallest. In these conditions half of all varieties kept their starch content unchanged (Saturna, Kardal and Kuras). The other half reacted also to good conditions by starch break down (Tomppa, Posmo and Seresta). The starch contents of these varieties were on an average 2.8 % lower after storage in a steady + 8 °C than it was after lift.

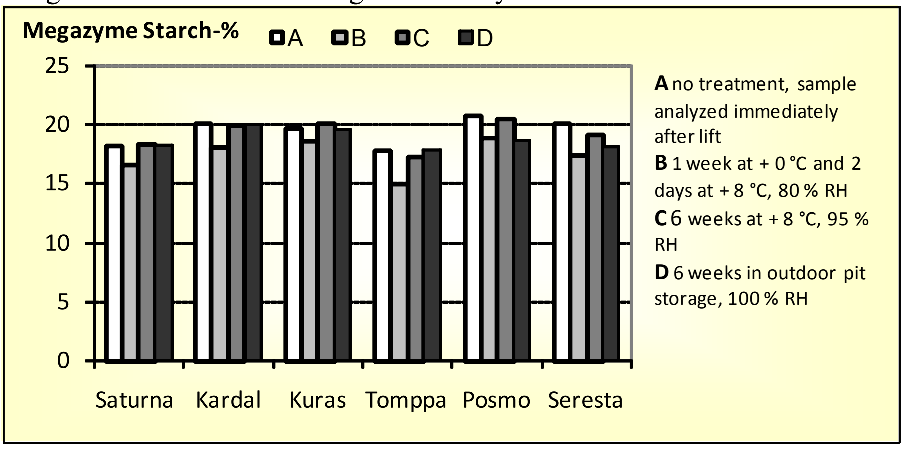

Figure 1. Starch contents (% FW), determined by Megazyme

The temperatures in pit storage vary between + 5 °C to + 14 °C (Fig. 2). The relative humidity was also high, 100 %. In these circumstances the starch contents of Saturna, Kardal, Kuras and Tomppa stayed at same level as they were at lift. On the other hand the starch contents of Posmo and Seresta were approximately 9,5 % lower than they were after lift (Fig 1).

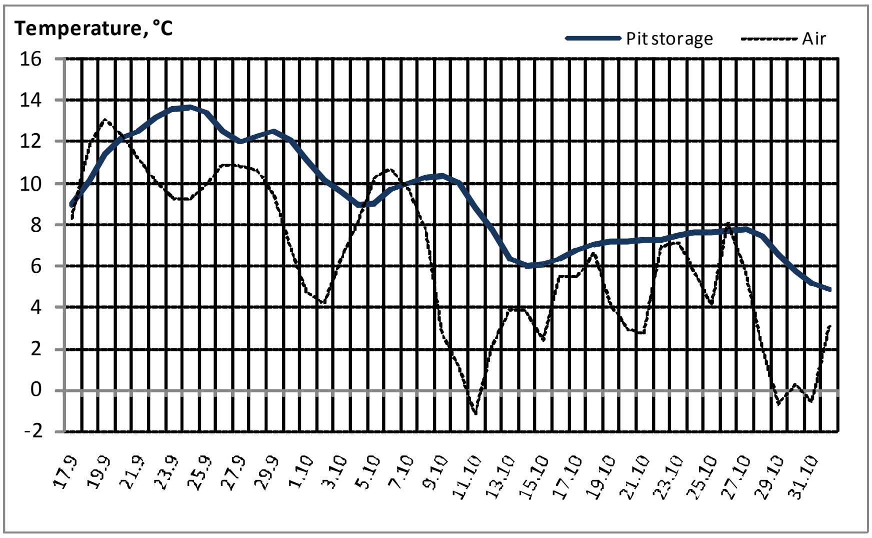

Figure 2. Weather and Pit Storage temperatures

Sugars

The sugar contents of all varieties were low after lift, but during cold storage plenty of sugars accumulated. After cold storage Tomppa and Posmo had the highest sugar contents and Saturna had the lowest (Fig. 3). The sugar contents of Saturna, Kardal and Tomppa were only a little bit higher after storing in a steady temperature of + 8 °C and pit storage than there were at lift. The sugar contents of Kuras, Posmo and especially Seresta also rose in these good conditions.

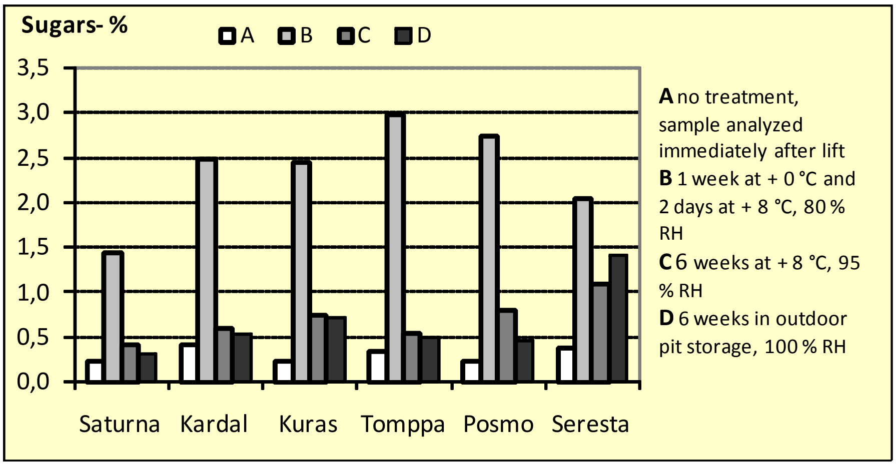

Figure 3. Sugar contents (% FW)

Starch, determined by specific gravity

Starch determining by specific gravity is based on the dry matter content of potatoes. In normal conditions a certain part of dry matter consists of starch and sugars. Specific gravity can not distinguish starch and sugars from each other, so it also counts high sugar contents as starch. This problem became apparent clearly in this study. In figure 4 you can see the result of this calculation: starch content (%) determined by specific gravity minus starch content determined by Megazyme has good correlation with total sugar content.

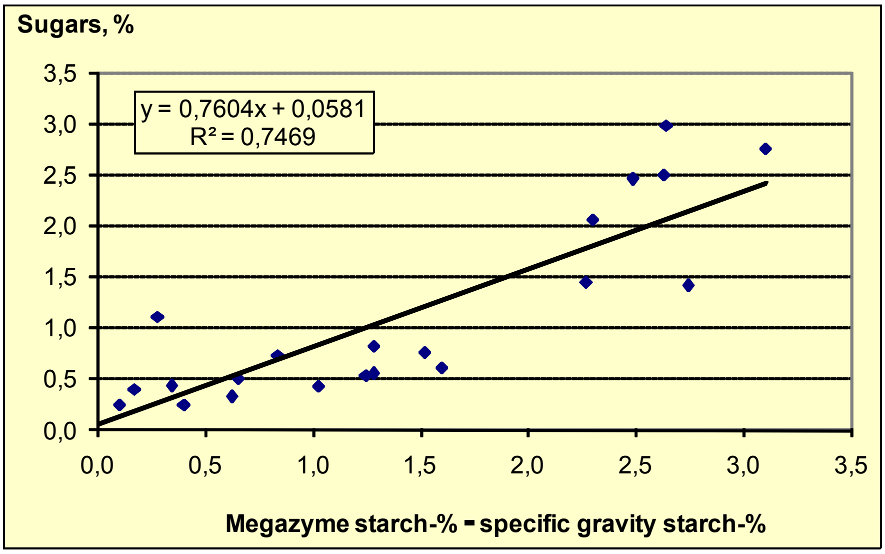

Figure 4. Starch content (%) determined by specific gravity minus starch content determined by Megazyme has good correlation with total sugar content

CONCLUSIONS

The loss that the potato starch production companies have noticed, is due to inaccurate specific gravity measurement. In cold weather conditions potatoes are predisposed to cold and part of the starch is transformed into sugars. Specific gravity can´t separate starch and sugars from each other. So specific gravity gives results that are higher than the true starch content is.

Varieties that are best suited to pit storing are Saturna, Kardal and Kuras. The starch content of these varieties stays at a good level, if the temperature in the pits is regulated. The sugar contents of these varieties, especially Saturna, stayed fairly low in cold conditions as well. Tomppa also is fairly good. It´s starch content stays at a good level and it´s sugar accumulation is slight if storage conditions are good. In cold conditions Tomppa´s sugar production was highest of all the tested varieties.

Less suitable for pit storing are the varieties Posmo and Seresta. With these two varieties starch break down took place also in good conditions.

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ASSISTORE - A DECISION SUPPORT SYSTEM FOR POTATO STORE MANAGEMENT

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AssiStore is a decision support software program to assist potato store managers. It has a web-browser interface which links to an MS Accessä database to drive the data logging and decision support functions. The program uses risk-assessment strategies to produce supplementary advice, which helps potato store operators in their management decision making. The key to the use of the software is to make regular assessments of crop quality on samples taken from store and this information is then fed into the program, along with environmental data (eg temperature), to generate updates on management requirements.

IMPROVING THE APPLICATION OF CIPC TO BULK POTATO STORES

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Introduction

Conventional thermal fog application of chlorpropham (CIPC) sprout suppressant to bulk stores, without fans operating, can result in uneven distribution of the treatment (McGowan *et al.*, 2007). This unevenness means that some areas in the store may receive little chemical, leading to earlier re-growth and the need for further applications.

Materials and methods

A modified application procedure, adapted from methods used in North America (Briddon & Jina, 2004), was evaluated in commercial stores over three years (2005/6 to 2007/8). In this procedure CIPC fog was re-circulated at low-speed (i.e. with fans operating at lower speeds than normally used for ventilation) through bulk potato piles. A solid formulation of CIPC was used for all modified applications in the trial. Results were compared with conventional application using a liquid formulation of CIPC (50% w/v CIPC in methanol) without recirculation.

In the modified treatment, fans were operated at low speed by connecting them to a variable frequency drive (also known as an inverter or a variable speed drive) to prevent the chemical depositing on fan blades and guards.

Before applications, in the modified treatment, airflow into lateral ducts was balanced. This was necessary as operating fans at lower speed reduces the air volume displaced and changes the static pressure distribution in the main duct. This will usually take the store's air handling system outside its normal operating limits (typically around 0.015-0.020 $m^3 s^{-1} t^{-1}$ and result in an unbalanced airflow. This would result in an uneven distribution of CIPC. At low fan speeds, most recirculated air is likely to pass through only a few laterals, usually those nearest the return route to the fan whilst relatively little air reaches the laterals further away. Partially closing shutters over laterals increases the static pressure in the main duct and forces more air down laterals further away from the fan.

The procedure for setting up a store for recirculation of CIPC was as follows:

Step 1: Adjusting the return path

The return path of air and fog from the store headspace back to the fan was set up to be as straightforward and unrestricted as possible by opening all internal doors and louvres between the fan and the store headspace. In addition, roof-space fans, refrigeration and humidification systems were either by-passed or switched off, as appropriate.

Step 2: Balancing airflow in laterals

An anemometer was used to check the airflow into laterals. An average air speed, across all laterals, in the range 2.0-4.5 m/s was used, with as little variability between laterals as possible. Where airflow rates were particularly unbalanced, it was necessary to reduce the outlet area of each lateral to around 50% of its normal size. This resulted in more even distribution of air, but was still unbalanced with airflow rates in some laterals being greater than others. To balance the system completely, lateral openings with the highest airflow rates (usually at the fan end) were closed down further, to around 30% of their normal size, and laterals with lower airflow rates (usually further away from the fan) were opened to between 50% and 100% of their normal size. By increasing the outlet area of laterals with low airflow rates, and decreasing outlet area of laterals with high airflow rates, the system was able to be balanced to allow similar volumes of air and fog to be delivered to the crop above all of the lateral ducts.

Step 3: Application

After balancing laterals, CIPC was applied using a conventional thermal fogger. Fog was recirculated continuously during, and after CIPC application, until the fog had cleared.

Where possible, CIPC fog was introduced into the fan house, and the fans used to draw the chemical into the main duct. This assists mixing of fog with air and helped to ensure the delivery of CIPC followed the pattern of air distribution when the store was balanced (Step 2). If application through the fan house was not possible, the fog was applied directly into the main ventilation duct.

Changes in airborne CIPC concentration were determined after applications using methanol traps operated by air sampling pumps. Commercial unloading of crops was monitored and netted samples, located at store-loading, recovered for assessment of sprouting (25 tubers) and CIPC residue analysis (3 tubers). Netted samples were located in 27 locations per store, covering all three store dimensions.

Results and discussion

In the 2006/7 season, 1, 2 or 3 applications of CIPC, at a nominal rate of 14 ppm per occasion, were made to trial stores, covering a range of storage temperatures, storage durations and cultivars. For all stores, using both application methods, mean maximum sprout length occurred in the range 0.6-8.0 mm with a mean of 2.0 mm. CIPC residue values were in the range <0.1-5.0 mg kg^{-1} with a mean of 0.9 mg kg^{-1}.

Conventional applications resulted in sprouting in the range 0.6-8.0 mm, with a mean of 3.0 mm. The modified application procedure resulted in a narrower range of sprout lengths (0.6-4.5 mm) and a lower mean sprout length (1.7 mm).

CIPC residue values were also more variable following conventional applications (<0.1-5.0 mg kg^{-1}, mean 1.1 mg kg^{-1}) compared with applications using recirculation (range 0.1-3.8 mg kg^{-1}, mean 0.8 mg kg^{-1}).

In addition to improving sprout control efficacy and CIPC residue distribution, continuous recirculation of CIPC fog through bulk piles resulted in more rapid deposition of the chemical compared with conventional applications (Fig. 1). This allows earlier ventilation of stores following applications, which may be expected to have a smaller impact on processing quality (Daniels-Lake *et al*, 2005).

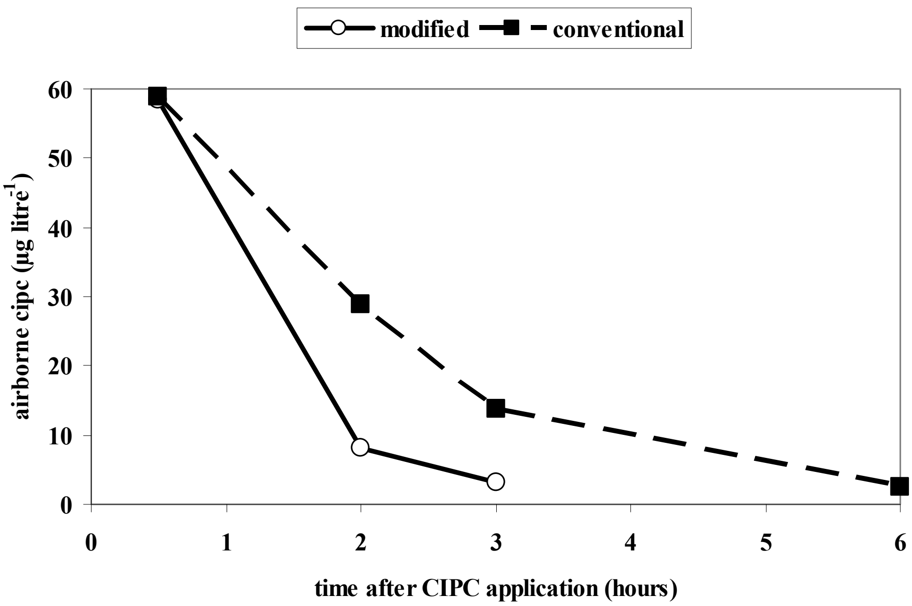

Fig 1: Change in airborne CIPC concentration after conventional application and a modified system using low-speed fog recirculation.

Low-speed recirculation of CIPC fog through bulk piles using variable frequency drives and adjustment of the outlet area of the main duct, to compensate for the reduced air volume, improves sprout control and residue distribution. The procedure offers a realistic and cost-effective means of improving stewardship of the chemical.

The process should also make good processing quality of potatoes easier to maintain, by minimising the duration for which crops are exposed to harmful by-products associated with the application process.

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EFFECTS OF RESTRAIN ON SPROUT GROWTH DURING STORAGE, STEM NUMBER AND SEED SIZE DISTRIBUTION IN SEED POTATO PRODUCTION

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Summary

The effects of Restrain (ethylene) on sprout growth during storage and subsequently on stem number and seed size distribution was investigated during 2004-05 and 2005-06. Restrain had significant effects: shorter sprouts, increased stem number per plant and increased tuber number per plant resulting in more medium sized seed tubers.

Experiment 2005-2006

The 2005-2006 experiment was conducted in mechanically refridgerated store rooms at Applied Plant Research (PPO) in Lelystad (Netherlands). Treatments consisted of two Restrain doses on eight (Dutch) potato cultivars.

Restrain dose	Storage temperature (°C)
0 ppm (untreated)	5-6
15 ppm	5-6
15 ppm	8-9

Potato cultivars: Agria, Annabelle, Baraka, Bintje, Carlita, Felsina, Kondor and Victoria.

Results

Sprout length at the end of the storage period, stem number in field and seed size distribution at harvest are presented in figures 1, 2 and 3 (data 2005-2006).

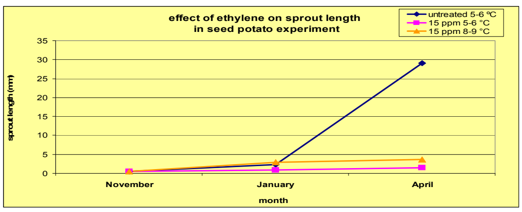

Fig. 1: Sprout length per ethylene treatment during storage period

Ethylene leads to short sprouts (figure 1).

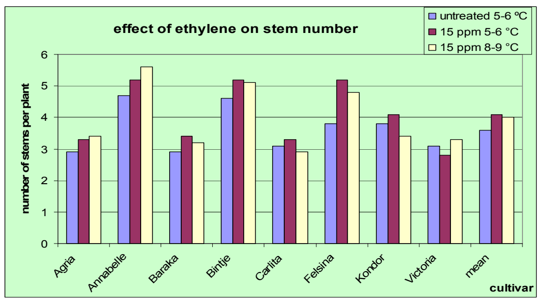

Fig. 2: Effect ethylene on stem number (20 June 2006)

Ethylene creates a shift towards more stems per plant in a number of potato cultivars (figure 2).

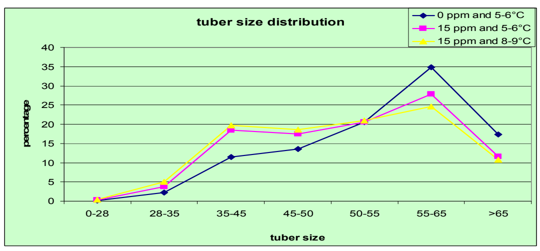

Fig. 3: Effect of two ethylene treatments on tuber size distribution

Ethylene creates a shift towards more medium seed sized tubers at harvest in a number of potato cultivars (figure 3).

Conclusion

The ethylene treatments in this experiment reduced sprout growth, increased stem number per plant and increased yield of more medium sized tubers in a number of the tested cultivars.

Funding

Restrain Company Ltd.
Norwich Road Colton Norwich Norfolk NR9 5BZ, UK www.restrain.eu.com

UTILISATION, PROCESSING AND QUALITY

SOME EXAMPLES FROM REGIONAL POTATO FOODS IN TURKISH CUISINE

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Keywords: potato (*Solanum tuberosum* L), regional foods, recipe,Turkish Cuisine

Abstract

Potato (*Solanum tuberosum* L.) was introduced in Turkey around 1850's, has not developed quickly in the beginning because its nutrition value was not well known. But nowadays, as in other parts of world, its nutrition value was known and human beings started to benefit from it as various types of food in our country. Tuber contain significant concentration of vitamin C and essential amino acids. Potatoes are either consumed directly or processed to give products such as chips and French fries, mashed and canned potatoes. In this paper only mentioned from some examples of regional potato foods in the Turkish Cuisine and explained food recipe of them.

INTRODUCTION

Potatoes knolls are consumed in the world cuisine as they are, or together with other vegetables or meat and fish meals, in various forms, or also processed, in the form of chips and finger potatoes. Potatoes, the knolls of which contain carbohydrates in form of starch, proteins, vitamins, and important nutrients such as Fe, can be mixed to the flour of bread in a ratio of 3-5%, so that the getting stale of the bread will be postponed, and its taste improved (Arıoglu, 2002). A potatoes knoll of 100g contains a minimum of 7% of the vitamins, 10% of iron, 20-50% of vitamin C, 10% of vitamin B_1, 3% of the energy needed by the human body every day (Burton, 1974). Potatoes, which is a nutrient for people of any age, is a source of food for the consumption of sick people, for diet, and for consumptions outside diets; it has a high degree of nutrients, can be digested easily, and does not tire the stomach (Otles and Akcicek, 2002).

In Turkey, which is made of seven different geographical regions, many different produce is obtained due to the climate factors. This diversity has brought forth a very rich life and cuisine cultures. As the cuisine cultures change, depending on the regions, there are also distinction in the same region (Artun, 1998). Potatoes, which has a history of approximately 150 years in Turkey, is consumed differently, depending on the region it is produced, and on the other food products that are consumed in the regions. For instance, in the cuisine of the Black Sea Region, where the fish and vegetable consumption is high, some of the famous dishes include "Potatoes with anchovy, "Fried potatoes", "muhlama", while in Sivas and Kayseri, which are located in the Central Anatolian Regions where the consumption of cereal products and red meat is higher, the dishes "Velibahk", and "Gubate" of Caucasian immigrants which are prepared with wheat flour and boiled potatoes are consumed. The baked potatoes which is offered to guests in the Eastern Black Sea Region, the "potatoes salad" which is made almost everywhere in the country by mixing boiled potatoes, parsley, peppermint, onions, olive oil, and various spices, the "sour potatoes", "humus potatoes" "Harran Towers" in our Mediterranean and Eastern Regions are among the consumed dishes. The Turkish cuisine includes many dishes such as "pressed potatoes, potatoes in embers, potatoes flatbread, potatoes tirilli, potatoes balls, potatoes casserole, potatoes soup, potatoes puree, potatoes with yoghurt", the main ingredient of which is potatoes (Otles and Akcicek, 2002).

This article includes the recipes of the potatoes dishes which were obtained from our research on "Regional Potatoes Dishes in Turkey", which is still continuing. We have focused in our research especially on the eating habits of the consumers and producers living in villages, and on regional dish recipes. Areas such as restaurants and fast consumption locations have not been included in this research. Data related to the topic has been obtained by personal interviews with the consumers in each region.

Some examples for the regional potatoes dishes from the Turkish Cuisine: The recipes have been prepared for 4 persons.

Fried Potatoes
Ingredients:
1 kg potatoes
1 bundle of parsley
1 middle sized leek
3 scallions
2 tablespoon of curd
1 teaspoon of red pepper flakes
1 teaspoon of black pepper
2 dessert spoon of salt
½ tea glass of vegetable oil
Regional name: patates tava

Preparation: The potatoes which are boiled in salt water are peeled and thoroughly mashed. Finely cut parsley, leek, scallion, red pepper flakes, black pepper and curd are added to it and they are thoroughly mixed. 1 dessert spoon of vegetable oil is added to a Teflon pan and heated. The mixture is placed onto the pan to a thickness of 1-2 cm. It is fried on a small flame, by turning it upside down, until both sides become pink, it is taken off the fire, divided into 4 parts, and served. This dish, which is prepared in the Black Sea Region, is consumed normally hot, with roasted pickle, which is a regional dish, or with Ayran (shaked yogurt)

Potatoes with yoghurt
Ingredients:
1kg potatoes
500g red meat with low fat
1 glass of chickpea
3 glasses of filtered yoghurt
1 egg
1 tablespoon of flour
1 teaspoon of salt
1 dessert spoon of dried peppermint
2 tablespoons of vegetable oil
a sufficient amount of water
Regional name: yogurtlu patates

Preparation: The chopped meat and the chickpeas which have been left in water during the previous night, are added salt and water and boiled thoroughly. The peeled and diced potatoes are added and cooked until they soften. After the yoghurt, eggs and flour are mixed together, they are added to the meat, chickpeas, and potatoes mixture on the low flame, while continuing to stir it. After they are boiled for approximately 2-3 minutes more, they are taken off the fire. They are served with heated oil and dried peppermint on top of them.

Harran Towers
Ingredients:
1kg middle sized white potatoes
50g tomato paste
1/2 tea glass of olive oil
1 dessert spoon of salt
1 teaspoon of black pepper
1 glass of water
Regional name: Harran kuleleri

Preparation: The potatoes are washed, slices into two pieces, and lined in an oven tray with their sliced sides looking down. Water, tomatoes paste, salt, black pepper, and oil are mixed and poured onto the potatoes. The potatoes are left in a stone oven at approximately 200-250 Celsius, until they are baked.The name of this dish from the Urfa region was based on "Harran", which is an ancient settlement, because it looks like small towers.

Potatoes salad with yoghurt

Ingredients:

1 kg potatoes

6-8 carrot

2-3 tomatoes

2-3 fresh onions

5-6 stalks of parsley

2 clove of garlic

1 glass of filtered yoghurt

1 tea glass of olive oil

1 dessert spoon of salt

1 teaspoon of black pepper

1 teaspoon of red pepper flakes

As a garniture: 6-7 leafs of fresh peppermint, 1-2 stalks of dill

Regional name: yogurtlu patates salatası

Preparation: The boiled potatoes are mashed with a fork while still warm. Salt, black pepper, and red pepper flakes are added to it. Yoghurt and mashed garlic is mixed in a deep container. This mixture is added to the potatoes mixture and they are all mixed slowly. This mixture is poured inside a glass tray of a depth of 5-6cm, and its top is smoothed out. The finely sliced carrots are cooked in ½ tea glass full of olive oil for 5 minutes, while stirring them all the time, and then left to cooling. Meanwhile, the tomatoes which are peeled and are grated, are cooked in ½ tea glass full of olive oil for 2-3 minutes, while stirring them all the time, and then left to cooling. The tomatoes are poured as a thin layer onto the potatoes mixture which is placed into the tray, and the carrots are poured on top of them. They are served cold with a garniture of finely sliced fresh onions, parsley, peppermint, and dill. This is a light dish, which is consumed in the Aegean region. It is usually preferred as an appetizer and salad.

Potatoes muhlama

Ingredients:

1 kg potatoes

½ tea glass of vegetable oil

3-4 stalk of fresh onions

1 teaspoon of red pepper flakes

1 teaspoon of black pepper

1 teaspoon of cumin

1 dessert spoon of salt

2 middle sized dry onion

2 eggs

Regional name: Patates muhlama

Preparation: The potatoes which are boiled in water and which have been added salt are peeled and diced. Finely sliced fresh and dry onions are cooked in vegetable oil which is placed inside a container, until they become pink. The slices potatoes are added onto the onions and they are cooked on a small flam for approximately 10 minutes while stirring them all the time. Two eggs are mixed in a different container. It is added to this mixture, while stirring continuously. After the eggs are done, they are taken off the fire, and served hot, after red pepper flakes, black pepper, and cumin is added and mixed to them. Potatoes muhlama is an easy-to-prepare dish which is consumed in almost any region, but which is cooked primarily in the Black Sea Region.

Potatoes with anchovy

Ingredients:

1 kg potatoes

1kg anchovy

2 middle sized carrots

3 middle sized dry onion

3-5 green pepper

1-2 red pepper

½ tea glass of vegetable oil
1 tea glass of hot water
2 dessert spoon of salt
Regional name: Hamsili patates

Preparation: The carrot, potatoes, onion, green and red pepper are diced, vegetable oil is added to them and they are cooked on a fire with a middle flame until the freshness of the vegetables disappears, stirring them slowly all the time. Salt and hot water is added to it. It is then cooked with a small flame until the water has completely evaporated. Half of the mixture is spread onto the oven tray and the anchovy which have been cleaned, the fishbone of which have been taken out, and which have been added one dessert spoonful of salt are lined on top of it. The other half of the potatoes mix is spread on top of the anchovy. 1 tablespoon of vegetable oil is poured on top of the mixture and it is left in the oven at 170 degrees until it is done (approximately half an hour). It is served hot, with finely sliced parsley spread on top of it. Depending on the preference of the consumer, red pepper flakes, black pepper, cumin, and garlic is added to the potatoes mixture, and the anchovy is seasoned with lemon.

We hope you like this foods. Bon appetite…

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COLD STORABILITY OF PROCESSING POTATOES

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Introduction

Special strategies are necessary to store large amounts of potatoes in such qualities, which allow the production of high quality products also after long term storage in spring or summer time. With regard to cold sweetening, most of those potatoes are stored under elevated temperatures (8-10°C) to prevent an excessive sugar accumulation. Thereby, the time interval until dormancy break is significantly reduced in comparison with a cold storage and as a result anti-sprouting chemicals are needed. The most popular among them is chloropropham (CIPC, chloro-isopropyl N-phenyl carbamate). With respect to CIPC residues and a never ending debate about acceptance of consumers and licence of authorities, efforts were undertaken to develop new cultivars without any cold sweetening. Contrary to GMO developments with enzyme blockade on a molecular level [Sowokinos, 2001; Sowokinos, 2002], German potato breeders used conventional breeding techniques to search for correlation outliers [Putz, 1997].

Since a couple of years, the Federal Office of Plant Varieties has included a special testing for so called "4°C-cultivars" in the official test programme: Samples are stored at 8° and 4°C for about six months, analyzed according to sugar concentration, and processed to both crisps and French fries on a semi-technical processing line of the MRI in Detmold. Next to the test samples, also an assortment of well accepted cultivars is treated in the same manner to get benchmark data. To give a summary of the individual results of the last decade, single data were compiled to indicate any trends of the breeding progress.

Results

Measured values of the survey are pointing out a high variability of individual data, both for reducing sugar content and quality scores of products. The reference samples had at least a variance of values comparable with that of breeding lines, but the range of values was smaller at the references (Table 1 to 3).

Tab. 1: Quality parameters of reference cultivars; average and min/max values of the period between 1998 and 2006.

Sampling time	Dry matter content (%)			Reducing sugars (mg/100 g FW)			Crisps score (1 – 10)			French fries score (1 – 9)		
	average	min	max	average	min	max	average	min	max	average	min	max
Harvest	25.8	24.3	28.4	61.4	23.8	108	7.91	5.33	9.33	6.63	6.28	6.85
Storage	26.8	25.7	30.3	77.7	35.5	152	6.87	4.83	7.88	6.07	5.36	6.81
Cold Storage	26.4	25.3	29.6	200	75.2	437	5.58	4.33	7.28	5.05	4.34	5.98

Tab. 2: Quality parameters of crisping cultivars; average and min/max values of the period between 1998 and 2006.

Sampling time	Dry matter content (%)			Reducing sugars (mg/100 g FW)			Crisps score (1 – 10)		
	average	min	max	average	min	max	average	min	max
Harvest	26.0	24.2	28.5	42.8	29.5	75.3	7.75	5.54	9.33
Storage	27.1	25.5	29.8	76.9	20.3	209	6.48	3.92	9.17
Cold Storage	27.0	25.1	29.3	177	57.9	467	5.23	1.75	8.17

Tab. 3: ***Quality parameter of chipping cultivars (French fries); average and min/max values of the period between 1998 and 2006.***

Sampling time	Dry matter content (%)			Reducing sugars (mg/100 g FW)			French fries score (1 – 9)		
	average	min	max	average	min	max	average	min	max
Harvest	23.5	21.8	23.9	51.2	37.8	61.6	7.25	6.91	7.56
Storage	24.3	22.1	24.9	54.5	27.0	118	6.41	5.93	7.50
Cold Storage	23.7	22.5	24.1	162	72.8	251	5.68	5.04	6.67

Figure 1 indicates the quality of new cultivars, suitable for cold stores in relation to the references. Since 1998 a total number of 4 cultivars entered the National list of potato cultivars, which have a suitability for cold storage. With respect to the relatively low validity of sugar analyses in relation to crisps colour, Figure 1 presents colour values (lightness) in relation to the data of the references. A lower value means a worse quality profile in comparison with the references.

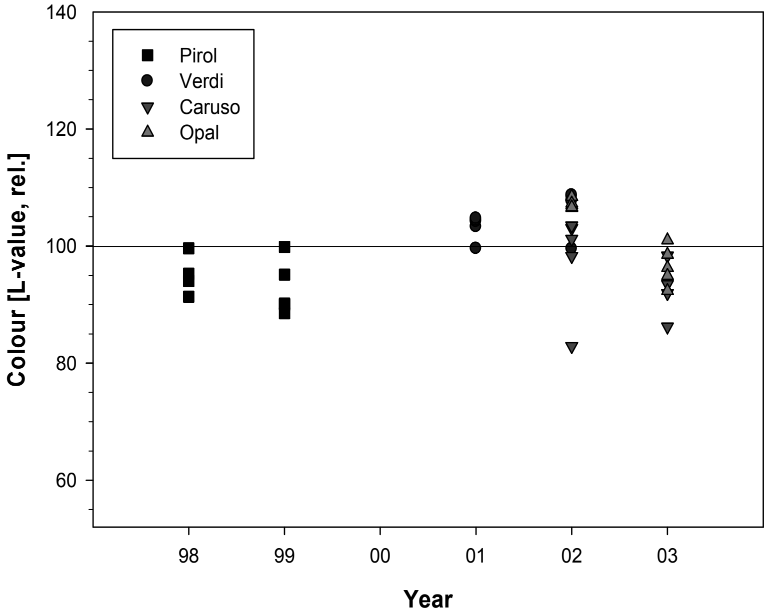

Fig. 1: ***Crisps colour of new cultivars, suitable for cold store, after a six months storage at +4° C, compared with reference values (= 100%).***

Conclusion

Cold storability of cultivars is suitable for processing as an important breeding target. First results of a conventional breeding programme demonstrate the principal realization. The industry is now invited to test these cultivars.

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VALORISATION OF THE POTATO PRODUCTIONS IN THE INDUSTRY : DUMPLING PRODUCTION FROM EARLY POTATOES

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Introduction

The potato European market is changing in the last years, influenced by the international commercial exchanges, the growth of the modern distribution systems and the variations of the consumer behaviour.

In this context, the Italian early potato productions show some problems in terms of export, because of the competition of the main Mediterranean Countries.

Different are the causes of such market loss: fragmentation of the offer, lack of a complete productive chain, standard quality of the product not corresponding with the demand expectation.

For these reasons, this typical cultivation is going to decrease more and more, that's why it is of prime importance to stop this trend by exalting the peculiar qualitative characteristics. These are influenced by the pedo-climatic conditions of cultivation.

In addiction, the way to exalt and transfer the property of a typical product into the industrial products could satisfy the demand of the consumers.

To this aim, the well known Sieglinde ancient German variety, successfully cultivated in Southern Italy, and in particular in the red soil of the Apulia region, is arousing interest by the industry for the production of "typical" potato product, on the basis of preliminary market research.

Favourable aspects is the harvest period of the Early cycle, starting in April, in the perspective of a wide period with available fresh potatoes for the industrial production .

To define the feasibility of the productive chain with early potato for industrial production, an industrial research has been carried out in collaboration with a food-industry involved in the production of potato dumplings, using fresh early potatoes of Apulia origin.

Material and Methods

The research has been carried out in the 2006 and 2007, in collaboration with Essedue Alimentare, industry in province of Treviso, specialised in the production of fresh potato derived (dumplings, potato pasta).

Some industrial tests have been carried out in the industrial production of fresh potato dumplings, testing the adaptability to the process, of the following varieties:

- Sieglinde, typical Apulia variety with little tubers and yellow pulp, high content in dry matter
- Annabelle, Dutch new variety used for Early cycle with long tubers and medium-low dry matter content
- Agria, one of the varieties of ware potato commonly used for dumplings production

All of the three variety were cultivated in Early cycle.

The technological process of dumplings provided the following listed phases:

- washing of potatoes
- steam cooking with skin
- separation of the skin by pressure and obtainments of a mash potato pulp
- mix of the ingredients (mashed potatoes 70%, flour of tender wheat, eggs, salt)
- moulding of the potato dumplings
- pasteurization, cooling and packaging under checked conditions

The following measurements were made in different moments of the production chain:

- dry matter and yellow index measured with colorimeter Minolta (L*,a*,b *) on the raw matter: (
- colorimetric parameters with Minolta Colorimeter (L, a , b *) on the dumplings
- water absorption (relationship between the weight of the potato dumplings before and after the cooking), colour of the water of cooking (0=trasparent, 2=cloudy), formation of sediment in the cooking water, sensorial analysis on the dumpling cooked in boiled water for about 3', until the climbing up of the dumplings. To obtain additional comparative parameters, a test sample of potato

dumplings of commercial origin was used , whose ingredients were rehydrated potatoes, starch of corn, flour of tender wheat, skimmed milk, citric acid, sorbic acid and aromas.

The evaluation of the sensorial characteristic of the cooked product was carried out through a panel test involving 8 tasters, using the QDA method (Quantitative decriptors analysis) (Van Marle, Van De Vuurst De Vries, Wilkinson, Yuksel, 1997). The following rheological parameters were measured: texture (adhesiveness, stickiness, firmness) and flavour (typical flavours, earth flavour, off flavour). The attributes were evaluated on a 0–5 points unstructured line scale.

The parameters measured have been submitted to variance analysis.

The aromatic profile of the cooked potato dumplings by instrumental system was carried out using the SPME technique (Oruna-Concha, Bakker and J. M. Ames, 2001) used for the extraction of the aromatic compounds in the head space and injection in GC MS used for the determinations of the compounds (recognized by consultation of data banks).

The changes Vitamin C content was carried out on Sieglinde variety samples , from the crude potatoes until the cooked dumpling. The determination of such compounds was carried out by HPLC (Hancock et al., …..).

The oxidation product of AsA, L-deidhro-ascorbic acid, was measured with addiction of 5 mMs of Tris(2carboxiethil through)phosphine hydrochloride (TCEP).

Results

Fresh tubers

The qualitative component of the fresh tubers of the 3 variety showed differences in terms of dry matter content, that was higher in Agria and Sieglinde varieties, scoring respectively 22% and 20,1% of average values. The dry matter content of Annabelle was very low (15,50%). The dry matter parameter is carefully considered in the industry, for a better adaptability to the process.

The yellow index (b°), indicating the intensity of the yellow colour, was higher in Sieglinde (27,5) in comparison to Annabelle (24,3) and Agria (22,9). Potentially all the three varieties underlined superficial eyes, allowing easy separation of the skin from the pulp during the process.

Potato dumplings

The qualitative characteristics of the potato dumplings underlined also substantial differences.

The yellow index b* of the gotten potato dumplings, was higher in the Sieglinde samples, 38,4, in comparison to the potato dumplings produced with Annabelle and Agria, respectively 34,7 and 33,7, higher than the commercial samples.

The behaviour of the dumplings during the cooking processes was also different. In Particular, the potato dumplings of Sieglinde showed an higher water absorption during the cooking, with tendency to the swelling in comparison to the others.

The samples of commercial potato dumplings showed the formation of sediments in the cooking water, probably due to the presence of non native starches between the ingredients.

The sensorial analysis confirm the differences between the potato dumplings produced with the three varieties. In particular, Agria dumplings scored a better rheological property, with low adhesiveness and stickiness, higher firmness; the flavour profile was instead quite flat, in terms of taste, earth flavour and typical flavour.

Sieglinde, on the contrary, underlined lower technological parameters, with tendency to a greater adhesiveness of the potato dumplings, while the sensorial parameters showed better flavour profile.

All the dumplings samples made with fresh potatoes showed better flavour and taste profile, in comparison of the dehydrated potato of commercial samples, except that for the rheological parameter of the consistence.

The aromatic compounds extracted by SPME technique in the dumpling potato samples were in total 155. Considering the single samples, in the cooked potatoes were isolated 56 compounds , in the potato dumplings 69, in the commercial samples 112.

Similar was the classes of compounds listed in the cooked potatoes and dumpling potatoes, with increase of compounds (69 in the dumpling vs 56 in the potatoes), especially in the classes of alkenes, alcohols and aldheides.

Instead, the number of compounds in the commercial dumplings was wider, 112, derived from the different ingredients added in the mixture and presence of new category compounds (amine, esthers, thiophenes, alkaloids), that contributed to modify the typical potato aromatic profile.

Conclusions

The experiment shows that Sieglinde variety produced in Apulia region in Early cycle could be used for the production of potato dumplings at an industrial level. The product obtained could differentiate between the “conventional” for the higher aesthetics properties as the yellow colour and the organoleptic properties, such as taste and flavour profile.

Adhesiveness, and stickiness (technological properties) of the Sieglinde potato dumplings, in comparison to testing variety (Agria dumplings), are lower,. This parameters, in any case, can be corrected at industrial level to improve the rheology of the potato dumplings.

The analysis of the aromatic compounds of the potato dumplings underlines the presence of a well defined classe of mixtures derived from the potato and from new compounds that come during the process (mixing and cooking), but they are well distinguishable from potato dumplings produced without fresh potatoes.

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Tab. 1 – Merceological and qualitative characteristics of the three tested varieties

Variety	Dry matter content (%)	Yellow index (b)	Tuber shape	Deeply of the eyes(**)
ANNABELLE	15,50	24,3	long-oval	1-2
SIEGLINDE	20,10	27,5	long-oval	1
AGRIA	22,00	22,9	oval-long	1-2
Variance analysis	**	**		

(**) 0= eyes prominent eyes, 5= deep set eyes

Tab. 2 - Qualitative and technological characteristics of the potato dumplings produced by the three varieties, compared with the commercial sample

Thesis	Yellow index (b)	Water adsobtion (p/p)	Water cooking colour	Sediment in the cooking water
Agria dumplings	33,7	1,0	0,1	absent
Sieglide dumplings	38,4	1,2	0,4	absent
Annabelle dumplings	34,8	1,1	0,6	absent
Commercial sample	35,0	1,0	1,6	consistent
Average	**35,5**	**1,1**	**0,7**	
Varianca analysis	**	***	***	

0= trasparent; 1= medium cloudy; 2= cloudy

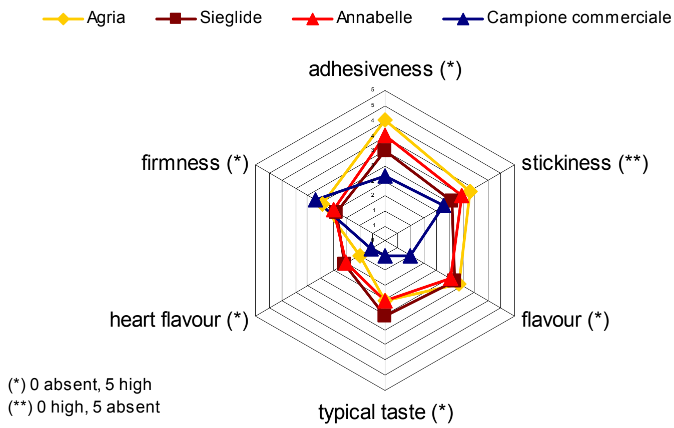

Fig. 1 - Results of the sensorial analysis of the potato dumplings compared

Tab. 3- Class and number of compounds extracted by SPME

compound classes	Cooked potatoes	Potato dumplings	Commercial dumplings
carboxylic acids	1	1	5
alkene	1	4	5
alcohol	3	8	3
aldehydes	17	22	18
Amine			1
Ketones	5	6	3
Quinone	1		
esthers	1	1	36
ethers	1	2	
Furans	2	2	2
Hydrocarbon	22	20	33
Pyrazine	1	1	
Terpenes	1	1	3
thiophenes			1
alkaloid			1
	56	69	112

WEIGHT, SUGAR FREE CONTENT AND POTATO TEXTURE EVOLUTION THROUGH DIFFERENT STORAGE PERIODS

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During the years 2004-2005 and 2005-2006 it was carried out a comparative study of weight, sugar free content and potato texture of storage potatoes in controlled and non-controlled storage conditions.

The sugar free content was controlled using a refractometer ATAGO 22000 WO3, the weight was studied with a scale Sartorious AG Göttingen 1100B-G, and the texture control was carried out using a penetrometer Bertuzzi FT 327.

Sugar free content presents an increasing tendency during storage period due to atmospheric conditions and tuber ageing. Texture values are positively related to relative humidity with an increasing period to January. From this moment to the end of storage period the tendency is the contrary. Weight presents a decreasing tendency through the storage period, up to 5% on initial weight.

These tendencies are very similar during non controlled storage conditions, but the values are bigger.

EVALUATION OF FLESH COLORED VARIETIES FOR SPECIALTY MARKET

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Key words: flesh colored potato, antioxidant capacity, tuber appearance, varieties evaluation

Abstract

The development of many human chronic and degenerative diseases is associated with oxidative stress. Diets rich in fruits and vegetables have been considered as excellent source of natural antioxidants. Potato tubers, in particular containing anthocyanins in flesh, can be their significant source. During three years totally 25 varieties were evaluated. Based on antioxidant capacity and tuber appearance blue-flesh varieties Blaue St Galler, Olivia and red-flesh varieties Mountain Rose and Herbie 26 are recommended for commercial production.

Introduction

The development of many chronic and degenerative diseases such as cancer, heart disease, neuronal degeneration has been associated with oxidative stress including its share in process of aging. Diets rich in fruits and vegetables have been considered as excellent source of natural antioxidants. Potatoes with their high per capita consumption and especially potato tubers containing anthocyanins are their significant source. Lachman (2000) and Brown (2005) consider polyphenols (phenolic acids, flavonols, anthocyanidins), ascorbic acid, carotenoids and tocopherols the major potato antioxidants. Friedman (1997) mentions chlorogenic acid as the major phenolic compound of white- and yellow-flesh potatoes. Harnly (2006) detected flavonoid quercetin in potato tubers. Burton (1989) mentions that petunidin, malvidin, delphinidin, pelargonidin, cyanidin and peonidin are the major anthocyanins of red- and blue-flesh potatoes. Sevcik (2005) detected cyanidin in variety Valfi. High concentration of antioxidant does not mean its high antioxidant activity or even bioavailability. Antioxidant capacity (AC) of foods is the most often determined by FRAP (ferric ion reducing antioxidant power) assay, ORAC (oxygen radical absorbance capacity) assay or TEAC (trolox equivalence antioxidant capacity) assays. AC determined by various assays could not be compared. Using of FRAP Halvorsen (2002) found more than eight-times higher AC in blue-flesh variety Congo compared to white-flesh variety Beate. By ORAC Wu (2004) determined portion of hydrophilic (H-ORAC) and lipophilic (L-ORAC) antioxidant capacity of white-flesh potato with different skins on total antioxidant capacity (TAC). H-ORAC was dominant and percentage of L-ORAC was less than 5% of TAC. Brown (2003) compared AC by ORAC and FRAP in tubers with white, orange, red and blue flesh. Tubers with white flesh had the lowest AC and some red- and blue-flesh breeding clones had AC more than three-times higher.

Materials and Methods

In three years 25 varieties were evaluated, 5 varieties in 3 years, 14 varieties in 2 years and 6 varieties in 1 year. In 2005 a set of 11 varieties, in 2006 a set of 23 varieties and in 2007 a set of 15 varieties was evaluated. Potatoes were grown in conventional production technology at planting distance 30 x 75 cm, fertilized by 100 kg N and P, K according to soil supply prior to planting and late blight control with 5 sprays during growing season in 2005, 2006 and 6 sprays in 2007. Tuber appearance was evaluated based on tuber size, shape and eye depth. AC was determined by TEAC assay using commercial set Randox TAS (radical ABTS ®) and expressed as μmol of trolox equivalents (TE) per gram. Total phenolics (TP) were determined by Folin-Ciocalteus method expressed as mg gallic acid equivalents (GAE) per gram.

Results and Discussion

In all three years variety Agria was grown as a control variety. Differences were found in antioxidant capacity between individual growing seasons, during storage at 5 °C the capacity mostly increased. It is consistent with results of Reyes (2004) and Lewis (1999). The lowest antioxidant capacity was found in yellow varieties Agria and Mayan Gold, while the highest one was regularly recorded for blue fingerling Violette and Vitelotte. Results of analyses in December 2007 are given in Table 1.

Table 1 Antioxidant capacity and total phenolics content in 2007 varieties set.

Variety	Antioxidant capacity in µmol TE/g	Total phenolics in mg GAE/g
Agria	4,10	0,65
Blaue Ludiano	9,92	1,29
Blaue St Galler	9,08	1,32
Farberkartoffel	7,89	1,00
Herbie 26	5,12	0,73
Herrmanns Blaue	9,93	1,30
Highland Burgundy Red	7,69	1,02
Mountain Rose	8,69	1,23
Olivia	8,25	1,23
Puca Quitisch	8,84	1,33
Red Cardinal	5,17	0,73
Salad Red	6,79	0,90
Valfi	7,15	1,00
Violette	9,63	1,65
Vitelotte	11,39	1,52

Further, tuber appearance was evaluated. Varieties Puca Quitish, Salad Red, Red Cardinal, Highland Burgundy Red, Vitelotte, Violette, Blaue Ludiano, Farber kartoffel and Unbekante Schwarze had unsuitable tuber shape or small-sized tubers.

Table 2 Relative comparison of potato varieties suitability for specialty market

Variety	Origin	Flesh color	Antioxidant capacity	Tuber appearance	Marketing potential
All Blue	United States	blue	(+)	+	(+)
Agria	Germany	yellow	-	+	-
Blaue Hindelbank	PRI Gene Bank	blue	(+)	+	(+)
Blaue Ludiano	PRI Gene Bank	blue	+	(+)	(+)
Blaue Mauritius	PRI Gene Bank	blue	(+)	+	(+)
Blaue Schweden	PRI Gene Bank	blue	(+)	+	(+)
Blaue St Galler	Switzerland	blue	+	+	+
Blue Congo	Finland	blue	(+)	+	(+)
British Columbia Blue	Canada	blue	(+)	+	(+)
Farberkartoffel	PRI Gene Bank	blue	+	(+)	(+)
Herbie 26	Germany	red	+	+	+
Herrmanns Blaue	Germany	blue	(+)	+	(+)
Highland Burgundy Red	PRI Gene Bank	red	(+)	(+)	(+)
Mayan Gold	United Kingdom	yellow	-	+	-
Mountain Rose	United States	red	+	+	+
Olivia	Germany	blue	+	+	+
Puca Quintisch	PRI Gene Bank	blue	(+)	-	-
Purple Fiesta	PRI Gene Bank	blue	(+)	+	(+)
Red Cardinal	PRI Gene Bank	red	(+)	(+)	(+)
Salad Blue	PRI Gene Bank	blue	(+)	+	(+)
Salad Red	PRI Gene Bank	red	(+)	(+)	(+)
Unbekannte Schwarze	PRI Gene Bank	blue	+	(+)	(+)
Valfi	Czech Republic	blue	(+)	+	(+)
Violette	France	blue	+	(+)	(+)
Vitelotte	France	blue	+	(+)	(+)

- poor (+) good + very good

Conclusions

In total 25 varieties were evaluated during three years. Based on antioxidant capacity and tuber appearance blue-flesh varieties Blaue St Galler, Olivia and red-flesh varieties Mountain Rose and Herbie 26 are recommended for specialty potato production.

Acknowledgement

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EFFECT OF FUNGICIDE TREATMENTS ON DRY MATTER AND SOME NITROGENOUS COMPOUNDS IN POTATO TUBERS

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Introduction

The use of chemical fungicides is still an important common practice in disease management to increase food production world-wide. However, very little work has been done to assess the changes induced by the fungicide in the chemical composition of different crops including potatoes.

Materials and methods

The research was conducted in two seasons (2000 and 2001) in Göttingen in Germany. In these experiments the two potato cvs Hansa and Milva and three different fungicides treatments were used. Various fungicides were used against *Phytophthora infestans*, which cause potato late blight disease. Three treatments (T) namely, T1 (control), T2 (Ridomil Gold + Tattoo + Acrobat plus + Dithane ultra + Ortiva + Amistar) and T3 (Shirlan) were applied in 2000. Also the same treatments were used with different fungicides combination in T2 (Ridomil Gold + Tattoo + Acrobat plus + Dithane ultra) in 2001.

Dry matter of the tubers was determined using fresh tubers whereas for other analyses freeze-dried material was used. Nitrogen concentration determined by LECO® CN-2000 nitrogen analyzer (USA) then the value was multiplied by 6.25 to get the value for crude protein. Nitrate was determined by SKALAR (Continuous-Flow-Analyzer, Dutch). The HPLC separation of fluorescent *o*-phtaldialdehyde (OPA) derivatives has been applied to the assay of free amino acids.

The purpose of the study was to assess the effects of the application of different fungicide treatments on potato plants on the changes of dry matter and nitrogen compounds namely crude protein, nitrate concentration and total free amino acids in potato tubers.

Results and discussions

As shown in Figure 1, the fungicide treatments increased ($P<0.001$) dry matter content of the potato tubers in cv. Hansa in 2000, whereas only treatment T2 decreased ($P<0.05$) the dry matter content in 2001. In the tubers of cv. Milva, it increased ($P<0.001$) when treated with T3 in 2000. Several investigators referred the accumulation of the dry matter in the tubers of treated plants with foliar pesticides to the increase of photosynthesis process in the leaves. In the study of Fidalgo *et al.* (1993), it was found that deltamethrin pesticide prolonged the life cycle of the potato plant i.e. delayed foliage senescence and increased the RuBisCo activity.

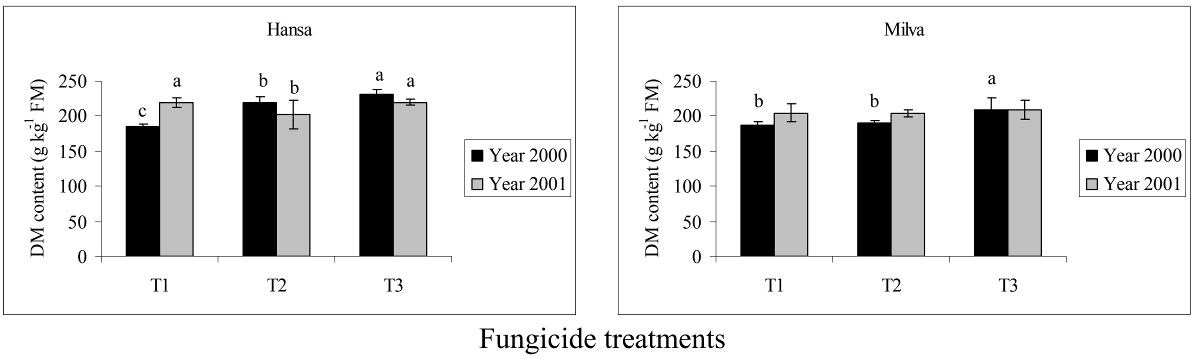


Figure 1 Dry matter content in potato tubers of cvs Hansa and Milva grown in 2000 and 2001 and treated with fungicides (different letters represent significant differences within fungicide treatments).

Also Gold *et al.* (1995) confirmed the delay of the plant senescence. Fidalgo *et al.* (2000) assumed that the accumulation of starch occurred mostly due to an increase in the supply of assimilates to the growing tubers as a result of the higher photosynthetic efficiency of the treated leaves. To interpret the decrease of dry matter by the application of some fungicides, Berger and Cwick (1990) as well as Pillonel (1993) considered that the toxicant produced by the application of a systemic fungicide inhibits respiration, photosynthesis and protein synthesis by inhibiting activity of NADH cytochrome "C" oxidases in the respiratory chain and accumulation of succinate and blocking of alternative pathway of respiration.

Crude protein in the tubers of cv. Hansa in 2000 decreased (P<0.05) as a result of fungicide application (Figure. 2).

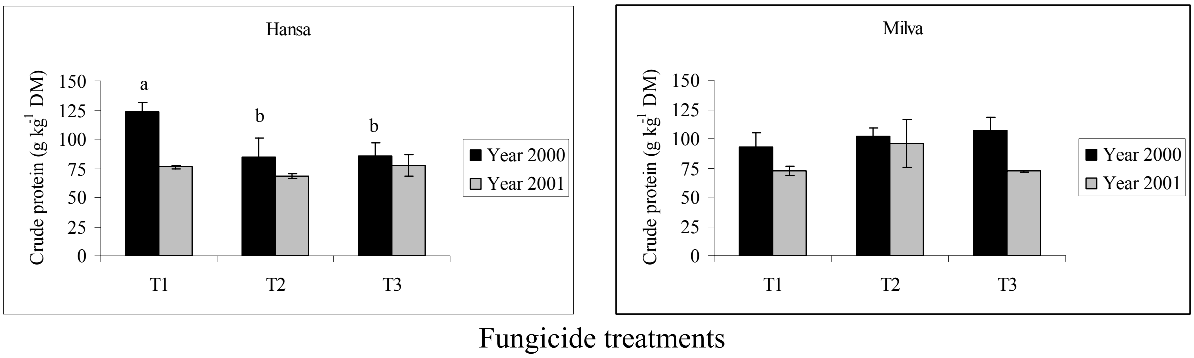


Figure 2 Effect of fungicide treatments on crude protein in potato tubers of cvs Hansa and Milva grown in 2000 and 2001 (different letters represent significant differences within fungicide treatments).

Nitrate concentration decreased significantly in the tubers of both cvs in 2000 with fungicide treatments (Figure 3). The significant reduction might be due activation of enzyme nitrate reductase caused by fungicide application as suggested by Glaab and Kaiser (1999) who concluded that Kresoxim-methyl fungicide caused an additional activation of nitrate reductase and prevented in part the degradation of the enzyme protein. Furthermore, the activation of the reductases may be due to the presence of some minerals in fungicides such as Mn or Zn.

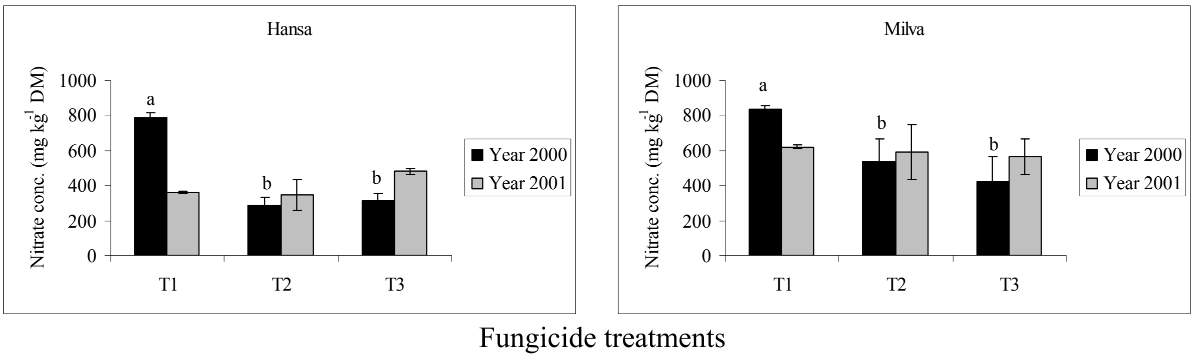
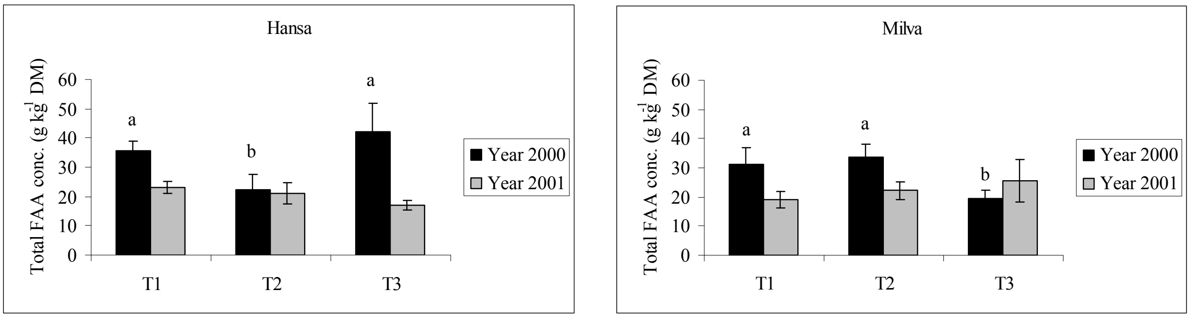


Figure 3 Influence of fungicide treatments on nitrate concentration in potato tubers of cvs Hansa and Milva grown in 2000 and 2001 (different letters represent significant differences within fungicide treatments).

In 2000 only, the total amount of free amino acids in the potato tubers of cv. Hansa decreased (P<0.05) when treated with T2. Nevertheless, in the tubers of cv. Milva it decreased (P<0.05) when treated with T3 (Figure 4). In 2000, most of the individual free amino acid in both cultivars decreased when treated with T2 or T3. In 2001, all free amino acids of cv. Milva did not modify significantly after the fungicide application. However, some of them in cv. Hansa changed.



Fungicide treatments

Figure 4 Total free amino acids concentration in potato tubers of cvs Hansa and Milva grown in 2000 and 2001 and treated with fungicides (different letters represent significant differences within fungicide treatments).

Many essential amino acids decreased as a result of fungicide application. This is may appear as an adverse nutritional sign since the nutritive value of protein and food products depends on their amino acids composition and essential amino acids balance. Generally increment of free amino acids considered as a sign of stresses. Furthermore, it has been reported that plants treated with fungicides may suffer from chemical stress (Siddiqui *et al*., 1997). Thus, the obtained increase in some free amino acids, mainly in cv. Hansa such as MET, TYR, ASP and GLU in 2000 with T3 as well as SER, LEU with T2 in 2001, might have a beneficial effect to face the toxic and undesirable impacts of pesticide on the treated plants. Like most of free amino acids, PRO researched here was mostly decreased after fungicide application. This indicates that plant may did not face any type of stresses as a result of the fungicide application.

Concluding summary

In general the dry matter content increased, crude protein, except in cv. Hansa in 2000, did not changed, nitrate concentration decreased and total free amino acids either remain unchanged or decreased by the fungicide treatments.

The application of fungicides guarantees disease control as well as improves growth of the crop plants which leads to high yield. The biochemical parameters studied contribute in varied manner to the quality and nutritional aspects of the potato tubers.

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VIROLOGY

THE STRUCTURE, ABUNDANCE, DOMINANCE AND CUMULATIVE VECTOR INTENSITY OF APHIDS ON SEED POTATO CROP (BRASOV 2002-2007)

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ABSTRACT

Among the pests infesting seed potato crops, aphids are the most virulent insects as virus vectors. The paper present the activity (structure, abundance, dominance and cumulative vector intensity) during 2002-2007 of aphid species and of main viruses vectors caught from Brasov seed potato crops. The most important species are: *Aphis fabae* (Scop.), *Aphis frangule* (Kalt.), *Brachycaudus helichrysi* (Kalt.), *Myzus persicae* (Sulz.), *Phorodon humuli* Schr., *Rhopalosiphum padi* L. Due climatic conditions, winged aphid population was different from one year to another. The most abundant aphid species were: *A. fabae, B. brassicae, A.frangulae,* and *Aphis sp.,* On the entire vegetation period, vector intensity presented high variation from one year to another. The highest variations were registered at the beginning and ending of potato vegetation period and shows the necessity and importance of early monitoring the aphid flight on a regional basis.

Key words: aphid, potato, structure, dominance, abundance, cumulative vector intensity

INTRODUCTION

Aphids are the insects of greatest economic importance on seed potato crops. When abundant, aphids are able to cause a large number of physiological changes in their host plant and in many case can reduce plant productivity.Aphid feeding is responsible for most of direct damage, which arises because of nitrogen and carbohydrate removal and injection of physiologically active substances in saliva. Indirect damage is mainly attributable to aphid excretion. Honeydew falling on leaf surface may cause decreases in light use efficacy and maximum rate of photosynthesis and increase in the rate of leaf senescence.

Six aphid species are most common on potatoes: the peach-potato aphid - *Myzus persicae* Sulz., glasshouse and potato aphid - *Aulacorthum solani* (Kalt.), potato aphid - *Macrosiphum euphorbiae* Thom., buckton aphid - *Aphis nasturtii*-(Kalt.), black bean aphid *(Aphis fabae* (Scop.) and *Aphis frangulae* Kalt. Other species as virus vectors not feeding on potato are: *Acyrtosiphon pisum* (Harris) *Brachycaudus helichrys* (Kalt.) *Phorodon humuli* Schr., *Rhopalosiphum padi* L., and about 40 different species

Viruses can lead to excessive yield loss in potato because they cause infectious diseases which are transferred with the seed tubers to the progeny crop. Yield loss can rise to more than 80%, depending on aphid species involved, type of virus, the potato variety, the rate of infection and growth conditions (Cancelado et. al., 1979). In Romania yield loss produced to plants secondarily infected with Potato leaf roll virus (PLTV) can rise to 53%-81% and to 33%-89% with Potato virus Y (PVY) (Cojocaru, 1987). European potato seed producers have long relied on monitoring of aphid flights for making decisions on when to top kill potatoes.

The researchers were carried out between 2002-2007 in Brasov. The site was situated to National Institute of Research and Development for Potato and Sugar Beet and the goal was to evaluate the structure, abundance, dominance and cumulative vector intensity of aphid population caught on potato crops. Knowledge on aphid species flight are indispensable for the development of a control strategy against vectors of virus diseases.

MATERIALS AND METHODS

The present work has several goals: to study the composition of aphid population caught on potato crops from 2002 to 2007; to determine the abundance and dominance of aphid population and of virus vectors also; to determine the cumulative vector intensity (vector abundance and their ability to transmit viruses) of aphids on potato crops.

Winged aphid monitoring was carried out by two Moericke yellow traps (round, metallic, 10 cm., deep, and inside painted yellow) installed at a height of 0.70 m., on the experimental area. Aphids were collected each morning, at the same hour, separated from other insects, identified and stored in glass vials containing preserving liquid (2 volumes of ethyl alcohol 90%, and 1 volume lactic acid 75% w/w) Eastop and van Emden, 1972). Aphid identification was based on the keys described by Taylor (1981), Műller (1975), Jacky and Bouchery (1980), Blackmann and Eastop (1984, 1994), Remaudiere and Seco Fernandez (1990). Systematic and synonymies were based on Remaudiere and Remaudiere (1997).

RESULTS

For identification of aphid species, about 6164 winged aphids were caught between 2002-2007. Annual abundance of species (Fig. 1) emphasis the evolution of aphid population:

- 2002 were trapped 43 species (2171 specimens); 2003 - 42 species (1498 specimens); 2004 - 46 species (940 specimens); 2005 – 38 species (252 specimens); 2006 – 43 species (877 specimens); 2007 – 26 species (426 specimens).

Due to climatic conditions, there were great differences between the years. In 2002 and 2003 winged aphid populations were most abundant -2171 and 1498 specimens, comparatively with 2005-252 and 2007-426 specimens. On each year the dominant specie was *Aphis fabae* with a total of 2182 individuals (2002 – 221; 2003 – 857; 2004 – 569; 2005 – 6; 2006 – 337; 2007 - 137). The tenth most abundant aphid species were: *A. fabae, B. brassicae, A.frangulae, Aphis sp., Hyalopterus pruni, Aphis sambuci, P.humuli, Hayhurstia atriplicis, Cryptomyzus galeopsidis, Aphis craccivora. Myzus persicae*, the most effective virus vector has caught sporadically in 2002 (2), 2004 (3), 2006 (2), 2007 (4), and abundant in 2003 (29). One important virus vector *A.solani* was not caught the entire period of observation.

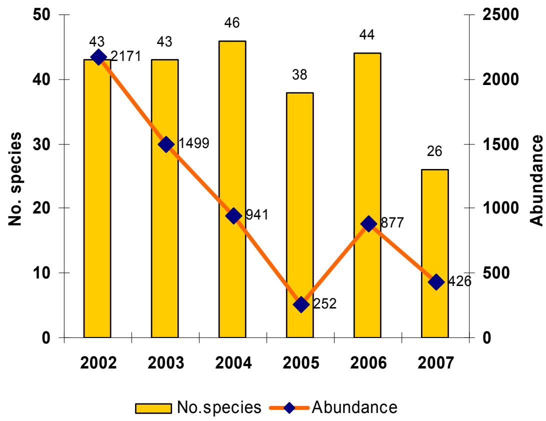
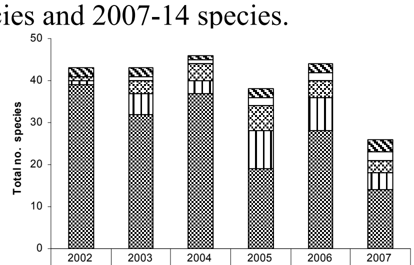


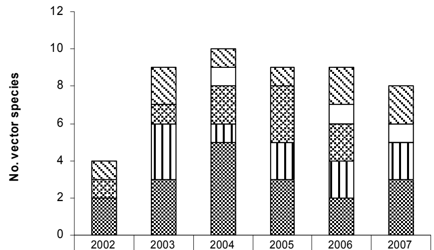
Fig. 1. The number of aphids caught on potato crop Brasov, 2002-2007

The abundance of aphid species caught (Fig. 2) was sub-divided on five different classes: **1-** (0-1%); **2-** (1.1-2%); **3-** (2.1-5%); **4-** (5.1-10%); **5-** (>10%). The most numerous species were comprised on class 1 (0-1%): 2002-39 species; 2003-32 species; 2004-37 species; 2005-19 species; 2006-28 species and 2007-14 species.



	2002	2003	2004	2005	2006	2007
Class 5 (> 10%)	2	2	1	2	2	3
Class 4 (5.1 - 10 %)	0	1	1	2	2	2
Class 3 (2.1 - 5 %)	1	3	4	6	4	3
Class 2 (1.1 - 2 %)	1	5	3	9	8	4
Class 1 (0 -1%)	39	32	37	19	28	14

Fig. 2. The structure of aphid species on potato crop Brasov, 2002 - 2007



	2002	2003	2004	2005	2006	2007
Class 5 (> 10%)	1	2	1	1	2	2
Class 4 (5.1 - 10 %)	0	0	1	0	1	1
Class 3 (2.1 - 5 %)	1	1	2	3	2	0
Class 2 (1.1 - 2 %)	0	3	1	2	2	2
Class 1 (0 -1%)	2	3	5	3	2	3

Fig. 3. The structure of aphid species vector of viruses on potato, Brasov, 2002 - 2007

Fig. 3 present the abundance structure of aphids vector of different viruses sub-divided on the same classes. In 2002, in spite of the most abundant aphid population (2171), only 4 species were identify as virus vectors. *A. fabae* belongs to class 5 (> 10%), *A. frangulae* to class 3 (2.1-5%), *M. persicae* and *P. humuli* to the class 1 (0-1%). In 2003, were identified 9 virus vectors. *A. fabae* and *A.frangulae* were the dominant species (>10%), followed by 1 specie from the third class (2.1-5%), 3 species from the forth class (1.1-2%) and 3 from the fifth class (0-1%).

In 2004, were trapped 940 specimens from which 10 species were vectors of different viruses. Two species were dominant - *A. fabae* and *A. frangulae*, and 6 species belongs to the fourth and fifth class.

2005 was the year with the lowest aphid population trapped - 252 specimens, from which 9 species were vectors. From total aphids, *A. fabae* was dominant. The other 8 species belongs to the third (2.1-5%), second (1.1-2%) and first class (0-1%).

In 2006, from 877 specimens, 9 were virus vectors. *A. fabae* and *A. frangulae* were dominant species, and 6 species were trapped sporadically. The second year with a lowest aphid population was 2007 when 426 specimen were trapped and 8 aphid species identified as virus vector. Three species were dominants –*A. fabae, Brevicoryne brassicae* and *A. frangulae* and 5 species were sporadically.

Between 2002-2007, the cumulative vector intensity of aphids weekly trapped on potato vegetation period (Table 1), recorded average values of 1.1-65.5. The highest value of virus vector meaningful distinct from the rest of weeks, was recorded on week 26. On the entire vegetation period, vector intensity presented high variation from one year to another. The highest variations were registered at the beginning and ending of potato vegetation period.

Table 1. Vector intensity of aphids on potato crop from Brasov (2002-2007)

Specification	Week										
	21	22	23	24	25	26	27	28	29	30	31
Vector intensity of aphids caught weekly											
Mean	7.7	10.5	5.2	19.0	23.1	65.5	26.5	10.9	19.4	3.6	1.1
Std. deviation	18.1	11.8	4.2	11.6	15.9	63.5	24.1	12.4	38.8	6.4	1.2
Minimum	0.0	0.2	0.1	6.2	5.2	7.0	1.9	0.4	0.1	0.1	0.2
Maximum	44.7	33.1	11.8	32.9	47.2	158.6	59.2	34.3	98.2	16.5	2.9
CV %	234.4	112.9	80.7	61.3	68.9	96.9	90.7	114.0	200.4	181.8	108.5
Cumulative vector intensity of aphids											
Mean	7.7	18.2	23.4	42.4	65.5	130.9	157.5	167.4	187.8	191.3	192.4
Std. deviation	18.1	29.5	29.7	34.5	34.5	62.0	76.9	86.6	94.0	96.9	97.6
Minimum	0.0	0.2	2.2	16.4	21.6	35.9	39.6	46.1	47.9	48.5	48.8
Maximum	44.7	77.8	81.8	106.0	123.0	198.5	257.4	291.7	297.1	297.6	298.3
CV %	234.4	162.1	126.8	81.3	52.6	47.4	48.9	51.4	50.1	50.6	50.7

The values of weekly cumulative vector intensity between May 19, and August 3, were separated using DUNCAN test on three sub-periods statistically distinct. Until the week 25, the average of cumulated value was 65.5, until the week 26 - 131.0, reaching an average of 192.4 until the week 31. The values of cumulative vector intensity presents high variation from one year to another also. The highest variations was registered on the first vegetation period (weeks 21-25), with CV over 80%. The high vector intensity which appears on certain years after potato emerged shows the necessity and importance of early monitoring of aphid flight on a regional basis. On the second vegetation period, the high cumulative vector intensity is characteristic for majority of the years.

The first spring migrants are difficult to detect because their occurrence is rare and in our case the trapping method with Moericke yellow water traps is not suitable for these kind of studies. A network of suction traps can provide effective early warning of aphid flight activity and to minimize virus spread in seed potato crops.

CONCLUSIONS

Controls of aphid-transmitted viruses in potato is difficult, complex and require preventive and therapeutic methods to avoid or minimize virus spread on seed potato crops. Timing and intensity of aphid flight, along with identifying the predominant aphids with some characteristics such us:

abundance, dominance, cumulative vector intensity are needed to develop an efficient integrated control program on a specific production area.

On Romanian seed potato areas aphid population is relatively high and comprise many species transmitted viruses.

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MANAGEMENT OF POTATO VIRUS Y IN SEED POTATO PRODUCTION IN FRANCE

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Introduction

Although viral diseases are usually well controlled in seed potato areas by rigorous production schemes, *Potato virus Y* (PVY, *Potyvirus* genus) poses a special threat and has become the main virus on potato crops in most areas in the world, as it is also the case in France. Besides traditional strains such as PVY^{N} and PVY^{O} strains, several variants of PVY have been reported such as PVY^{NTN} and PVY^{N}-Wilga which share higher virulence and agressivity on potato. The certification of seed potato requires 1) using efficient tools to detect all PVY isolates, and 2) evaluating the epidemiological risk associated with these PVY variants for the customer. Indeed, it is of special importance for seed growers and inspectors to have a good knowledge on the emergence of new variants which may induce milder symptoms on potato or on the contrary severe necrosis on tubers (PTNRD).

Material and methods

Every year, about 1,5 million tubers are planted in France in postharvest control to check the sanitary quality of seed lots. Leaves are then tested by ELISA technique with a mixture of polyclonal and monoclonal antibodies (INRA-FNPPPT) to have a large-broad detection of PVY.

Survey for PVY strains is done every year first by serology with specific (Mabs) antibodies (INRA-FNPPPT) allowing the differentiation between isolates of serotype-N (PVY^{N}, PVY^{NTN}) and isolates of serotype-O (PVY^{O}, PVY^{C}, PVY^{N}-W), then with molecular tests conducted partly at INRA and partly in the seed potato laboratories, with specific primers for the detection of PVY^{NTN} or PVY^{N}-Wilga isolates (Glais et al., 2002; 2005).

An evaluation of new detection tools is done on surveys. Since 2006, potato samples are sent to INRA to be characterized with SNP markers (Balme-Sinibaldi et al., 2005, Rolland et al., 2007) in comparison with classical detection methods.

About 120 seed lots are replanted every year in a national field to check the quality of the certification scheme and thus allowing comparisons between visual symptoms and laboratory results. In order to evaluate the biological significance of new variants and to show the symptomatology of PVY variants to seed inspectors and growers, a joint trial (FNPPPT-GNIS-INRA) has also been conducted, using mechanical inoculation of different PVY isolates on several cultivars (Amandine, Bintje, Charlotte and Nicola). A specific trial has been set up for PTNRD since 2005, consisting in indexing potato tubers of susceptible cultivars such as Nicola or Monalisa and planting them under net houses in order to evaluate the risk of necrosis on daughter tubers.

Results

Though there is no global increase in total PVY, results show a shift in PVY strains populations in France, with a general decrease in the serotype-O occurrence (which represents now in France about only one third of PVY strains, instead of 85% in the eighties) and a progressive increase of serotype-N. Within each of these serotypes, we observed a falling incidence of traditional PVY^{O} and PVY^{N} isolates with an increase in PVY^{NTN} and PVY^{N}-Wilga variants which are becoming more wide-spread. These results are rather similar to those previously described (Lindner, 2007, Van der Vlugt et al., 2007, Dedic et al, 2007, Glais, personal communication).

The symptoms induced on potato foliage showed a high variability, according to the viral strain and to the cultivar. However, PVY^{NTN} variants usually expressed rather severe symptoms similar to PVY^{O}, and on the contrary, $PVY^{N}W$ tended to express milder symptoms (mosaic, vein deepening). The strongest symptoms were expressed with the cultivar Bintje on the foliage (for all tested PVY isolates) and with the cultivar Nicola on tubers (PTNRD) with PVY^{NTN} isolates.

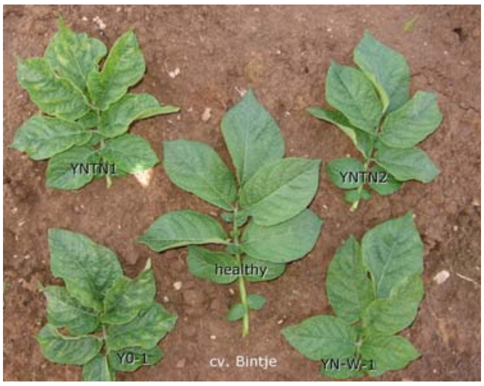

Figure 1: Example of the variability in symptom expression induced by different PVY isolates on the cultivar Bintje (primary infection)

For PTNRD, trials conducted during 3 years showed that even on susceptible cultivars, the average was only 10% of daughter tubers expressing tuber necrosis when seed tubers carried latent infection.

So, a better understanding of the factors involved in tuber necrosis is required, including the cultivar and the viral isolate but also the environmental factors.

These trials are conducted in parallel with the validation process of new detection tools (SNaPshot).

Conclusion

A close connection between research and certification experts is needed to develop reliable detection techniques and to adapt the tolerance for seed certification to the development of new viral isolates.

Concerning the PVY necrotic isolates, the development and the validation of new techniques which are related to biological properties, such as the Frit'N tests (Rolland et al, 2006), would be of special interest for the quality of seed potatoes and is a priority for joined research programmes between research and seed potato organizations.

Acknowledgments : This work is conducted in close cooperation with the INRA Bio3P-Phytovirus group (leaders : E.Jacquot and C.Kerlan) and the regional seed potato organizations (Bretagne-Plants, Comité Nord and Grocep).

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SOIL-BORN VIRUSES IN TATARSTAN

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Two soil-born viruses potato mop-top virus (PMTV) and tobacco rattle virus (TRV) have been identified in Tatarstan (Russia) for more then 20 years ago. Both soil-born viruses are most widespread in small farms and potato plots surrounding private houses. These potato growers occupy up to 90% of potato plantings in Tatarstan. PMTV is more widespread on thick soil and TRV on sandy soil.

We also have found PMTV transmitted by *Spopngospora subterranean* on potato minitubers in greenhouse. Simple and highly effective soil-born virus control measures have been used for potato multiplication in greenhouse condition.

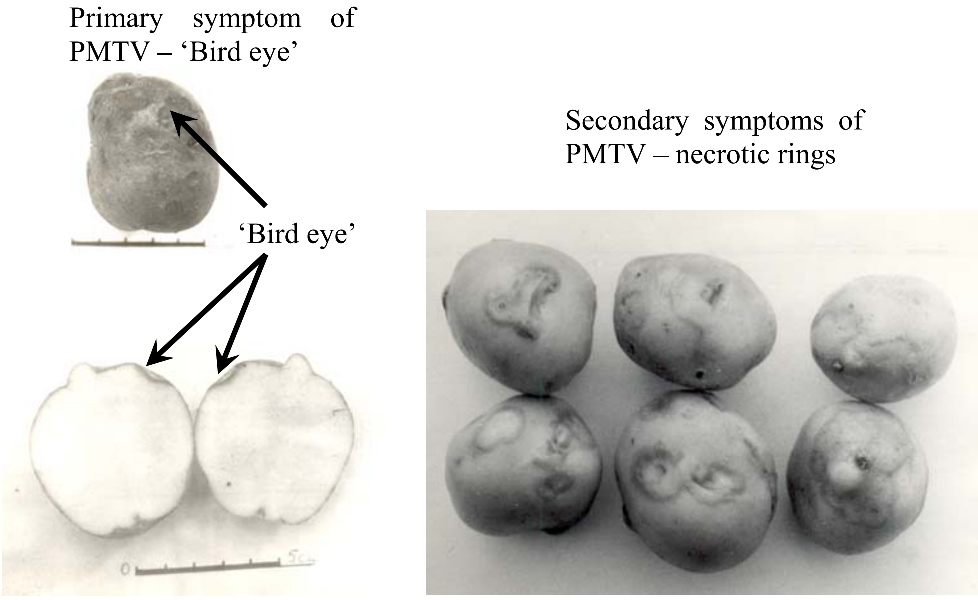

Figure 1. Primary and secondary PMTV symptoms on potato tubers.

We have detected 'Bird eye' symptoms on potato tubers in Russia until now known as a symptom of PMTV on *cv.* Renacimiento *Solanum tuberosum ssp. andigena* in Peru (Hinostroza and French, 1972). PMTV was detected almost in all potato tubers showed 'Bird eye' symptoms by using biological and ELISA tests.

'Bird eye' is a primary symptom of PMTV taking origin from inoculation by fungus-transmitter located in pustules. Necrotic rings are secondary symptoms can be seen in the next tuber generation obtained from virus infected tubers with primary symptoms (Jones, 1988). Necrotic rings can become visible also in the present year then virus carrying fungus-transmitter infects potato plant through root damage. 'Bird eye' appearance on potato tubers depend on storage condition and can case by high humidity and temperature jump.

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THE EFFECT OF SAMPLES INCUBATION MODALITY ON DETECTION OF PLRV BY ELISA TECHNIQUE IN LEAVES AND SPROUTING TUBERS

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Key words: potato, co-incubation, tubers, PLRV, ELISA

ABSTRACT

The results show a better identification of PLRV in leaves and sprouting tubers using the co-incubation sample and IgG-AP conjugate. In comparison with the classical method, the test safety and sensitivity increased. Testing leaves, the average values of OD at 405 nm was 5-6,6 times higher than those obtained by standard DAS ELISA method and using sap from sprouting tubers (dilution 1/10) this average was 1,5 times higher. The co-incubation sample and conjugate could save time and costs of seed indexation .

INTRODUCTION

The employment of high sensitive methods of detection and identification of nucleic acids, that allow for virus detection directly in plant extract, is still difficult for routine indexation of potatoes seed because of the high cost, complicated preparation of samples and highly trained personal needed to perform this kind of work (Leone et al. 1997, Nolasco et al. 1993, Schoen et al 1995, Spiegel and Martin 1993, Teverovsky et al. 1997 citated by Treder et al. 2005).

So, ELISA is the most commonly assay for detection of virus particles in potato tissues. A lot of researches were targeted to make modifications of this assay their purpose being to increase its performance or to enhance its detectivity (Van den Heuvel and Peters 1989, Martin R. H. 1990, Treder at al. 2005).

The detection level of the viruses and the rate of immunological reaction depend on which part of the plant is used for assay and on several physico-chemical factors like: temperature, diffusion of components in reaction (mixing), composition of buffers (Rek 1983, De Bokx 1987, Martin 1990, Treder at al. 2005).

The results presented in this paper show a better identification of PLRV in leaves and sprouting tubers (after natural break of dormancy) using the co-incubation sample and IgG-AP conjugate, together in the wells of the ELISA microplates. This method has been used by others researchers, in different conditions (Van den Heuvel and Peters 1989, Treder et al. 2005). Particles of IgG immobilized on the well surface are used to entrap virus particles, which bind conjugate particles at the same time, resulting in formation of multilayer structure of antibody-antigen-enzyme complex. This system allows binding higher amounts of virus and conjugate particles than occurs in regular DAS-ELISA (Treder et al. 2005).

MATERIALS AND METHODS

Potato material. All the biological material (healthy and infected) was obtained from the virus collection of our institute. We used 29 plants infected with two isolates (19 with the isolate Lusewitz and 10 with the isolate Braunshweig). The infection of this matherial was confirmed by using antisera from Bioreba (Switzerland). A pollahne press with smooth rolles was used for preparation leaf samples. For the tuber testing, the sap was extracted , diluted and dispensed directly into the plate using the extractor Microlab 500B/C **(**Hamilton**)** (Gugerli 1979, Rek 1983, Hill 1984). We tested sprouting tubers after natural break of dormancy, when the sprouts were 2-3mm long.

Antibodies and conjugates dilution as recommended by the manufactures 1:200 for Loewe (Germany) and 1:1000 for Bioreba AG (Switzerland).

Microplates- NUNC microplates were coated with antibodies for overnight incubation in the refrigerator.

DAS ELISA (V1) The analysis was performed following essentially the protocol described by

Clark and Adams (1977). We used 100 µl from each reactive solutions in each well of the plate.

In the other variant (V2, cocktail ELISA), all the steps were the same as DAS ELISA except that the sample and the enzyme conjugate IgG were added together and incubated overnight at 4°C. All experiments were repeated four times. Rinsed microplates were filled with substrate solution (p-nitrophenylphosphate) incubated 30, 60 and 120 minute and the absorbance values were estimated at 405 nm on PR1100 reader. The samples having A 405 values exceeding the cut-off (two times the average of healthy control samples) were considered virus infected.

RESULTS AND DISCUSSION

As shown in table 1, the sensibility of detecting PLRV in leaves was correlated to the incubation modality of the samples and the incubation time with substrate solution. In comparison with the classical method, the test safety and sensitivity increased. Testing leaves, the mean values of OD at 405 nm was 5-6,6 times higher than those obtained by standard DAS ELISA method.

Table 1. Detection PLRV in leaves by DAS ELISA* and COCKTAIL ELISA**

	Incubation time with substrate solution					
	30 minutes		60 minutes		120 minutes	
	V1*	V2**	V1*	V2**	V1*	V2**
Cut off	*0,065*	***0,084***	*0,072*	***0,113***	*0,097*	***0,171***
OD_{405nm}***	0.055±0,014	**0.278±0,119**	0.089±0,030	**0.536± 0,230**	0.162±0,063	**1.072±0,450**

x 5.0 x 6.0 x 6.6

*V1 – DAS ELISA
**V2 – COCKTAIL ELISA (co-incubation samples and IgG-AP conjugate)
*** – mean values of OD at 405 nm for four repetitions ± standard deviation

Significantly higher readings were obtained applying cocktail ELISA, this variant improving the detectability of potato virus particles.

Table 2 shows that the co-incubation sample and IgG conjugate gave positive reaction in 100% of the testing plants even after the shortest incubation time with substrate solution. When we used DAS ELISA, the percentage of infected plants grew with the time of substrate incubation from 37,5 to 82,8%, but the maximum percentage wasn't achieved not even after 2 hours despite the assays were made with plants sure infected. So, using the standard method, 17% of the samples didn't lead to positive reaction.

Table 2. Detectability of PLRV in leaves function on the samples incubation modality

Incubation time with substrate solution	Plants*** with positive reaction			
	V1*		V2**	
	Number	%	Number	%
30 minutes	10	37,5	29	100
60 minutes	18	62,1	29	100
120 minutes	24	82,8	29	100

*, ** - See explanations to table 1.
*** - Biological material used for leaves samples (29 plantes infected with PLRV). Experiments were repeated on four occasions, with the same results.

Testing sprouting tubers, the results showed a better identification of PLRV using the co-incubation sample and IgG-AP conjugate at all the sap's dilutions (table 3). Using the co-incubation sample with conjugate, for sap's dilution 1/10 and 1/20, the infected tubers were easily distinguished from negative controls. PLRV has been practically undetectable by DAS ELISA in tuber extracts diluted more than 20 times, while in the cocktail version, readings differentiate extracts from infected tissues, but not very clearly.

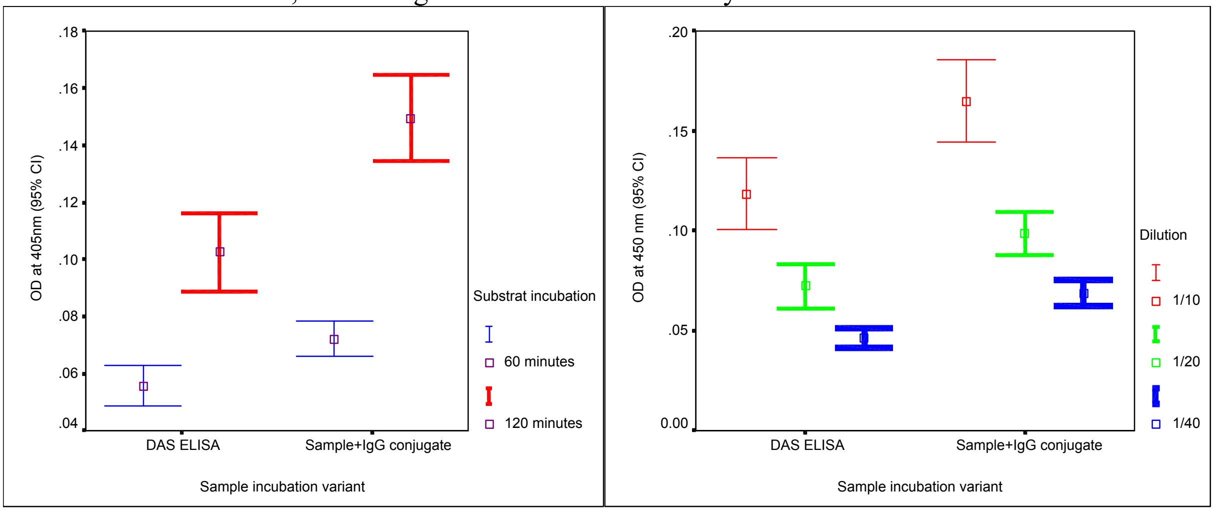
Table 3. Virus detection in sprouting tubers fonction on the samples incubation modality

Dilution of the sap	Incubation time with substrate solution(minutes)	V1*		V2**	
		Healthy	OD_{405nm} ± SD***	Healthy	OD_{405nm} ± SD***
1/10	60	0,025	**0,082 ±0,036**	0,031	**0,103±0,017**
	120	0,030	**0,155±0,060**	0,037	**0,227±0,042**
1/20	60	0.027	**0,050±0,014**	0,030	**0,065±0,007**
	120	0,036	**0,095±0.043**	0,038	**0,132±0,019**
1/40	60	0,026	**0,035±0,009**	0,031	**0,048±0,006**
	120	0,030	**0,058±0,017**	0,037	**0,089±0,014**

*, **.*** - See explanations to table 1.

The sap was extracted from the rose end of 29 sprouting tubers (after natural break of dormancy).

The figure 1 shows that the highest values of OD at 405 nm were those obtained by cocktail ELISA, when the tuber extract was diluted 10 times, after 2 hours substrate incubation. The mean values was in this case 1,5 times higher than those obtained by standard DAS ELISA method.



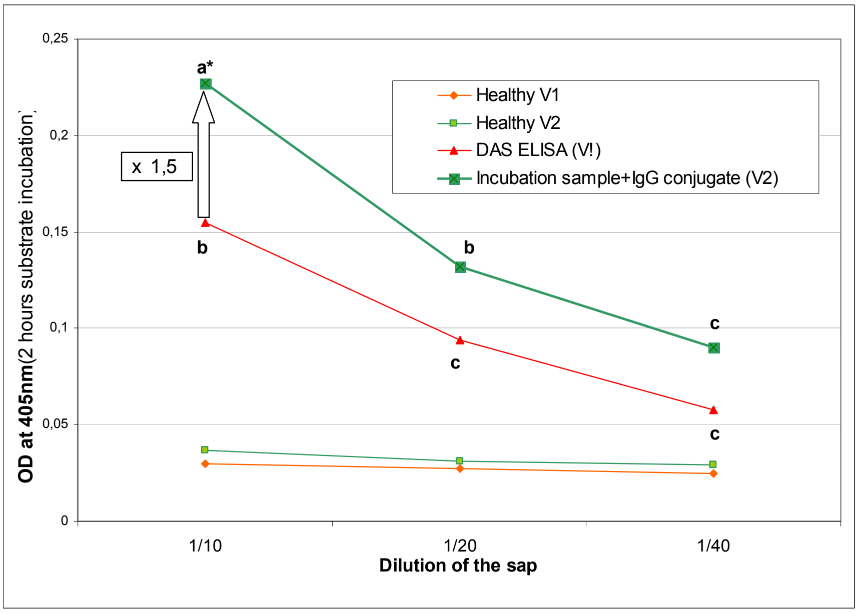


Figure 1. PLRV detection in sprouting tubers.

95% CI- 95% confidence interval of the difference.

*Values not followed by the same letter are significantly different (P=0,05) according to Duncan's test.

Experiments were repeated on four occasions. For further explanations see note of table 3.

The influence of the sample's incubation modality was more accentuate testing leaves than using sprouting tubers. Consequently, the results obtained in the detection of PLRV in leaves were better than in tubers (because of the low concentration of the virus in tubers).

About increased sensitivity, the co-incubation sample and conjugate could save time and costs of seed indexation (because of the elimination of two steps).

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VITRO CULTURE AND MICRO / MINI TUBERS

BEHAVIOR OF ROMANIAN POTATO VARIETIES CHRISTIAN AND ROCLAS ON MICROTUBERS PRODUCTION

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Summary

Due to a powerful international competition Romanian national system of seed potato production is confront with certain technically and economically compulsions. To solve these problems and rapid promote the Romanian potato varieties, INCDCSZ Braşov is engaged in realizing production of micro-plants, micro-tubers and mini-tubers.

Microtubers from Romanian varieties were obtained using potato micro-cuttings cultures on Murashige-Skoog medium enriched with Coumarin and Kinetin.

For inducing and growing of micro-tubers the cultures were maintained 8-10 weeks on darkness, to 18-20^0 C.

Key words: microplants, microtubers, *in vitro* tuberisation

Introduction

Researches from last period showed that microtubers (*in vitro* tubers) besides minitubers (nuclear tuber) have potential and can be integrated with success on seed potato production program (Khuri and Moorby 1996; Kim et al.1999; Struik and Wiersema 1999; Pruski K, 2003).

Microtubers production technique was described by researchers as a complex process, which can be influenced by a lot of factors.

Many researchers denote different growth regulators: benzyl aminopurine (Wang and Hu 1982; Hussey and Stacey 1984), 2-chloroethyl trimetyl ammonium chloride (CCC) (Tovar et al., 1985), coumarine (Stallknecht and Farnsworth 1982a; Dodds et al., 1988), jasmonic acid (Pelacho et al., 1993; Pruski et al. 2003).

There are many research results concerning the effect of culture media, photoperiod, temperature and genotype (Wang et al., 1994; Hoque et al., 1996; Zakaria 2003; Yeasmin 2005). Even the light is a very important factor for growing plants, the induction of microtuberisation is accomplished very well in dark conditions.

Owing to reduce laboratory production costs, many countries use microtubers on seed production program. Due to viral infection, production of certified seed potato is for Romania a major problem, and obtaining of microtubers is now a national priority. The researches had several important goals: founding in a short time an efficient method to obtain potato initial material free of diseases for new produced varieties, valuable from agronomic point of view and marketable.

Materials and methods

Initiation and tuberisation method is well known and it is applied on countries involved in seed potato production. For microtubers producing 2 steps were accomplished.

1. ***In vitro* cultures of microplants.** Healthy tubers, tested by DAS-ELISA technique represent the starting point for in vitro plantlets regeneration (assays drawn directly from tubers) from Romanian varieties Christian and Roclas.

Potato sprouts of 2 cm, grown on light and temperature of 18ºC, are drawn, disinfected and aseptically inoculated on test tubes (15/1.5cm) with 5ml Murashige-Skoog (MS 1962) medium, vitamins, sucrose 20g/l, ANA 0.5 mg/l, Phyto agar 8 g/l and pH: 5.7-5.8, adjusted before autoclaving.

After 3-4 weeks, depending on varieties, the sprouts regenerated new plantlets, which were micro-cuttings (5 uninodal cuttings/plant) with the possibility to obtain material free of diseases from each variety.

Uninodal cuttings were inoculated in jar (6/6 cm) containg 30 ml Murashige-Skoog medium. Each jar contains 10 uninodal cuttings.

Culture was place on growing room at 20º±2ºC (by day) and 18º±2ºC (by night) and a photoperiod of 16 hour light and 8 hour darkness.

2. *In vitro* tuberisation. After 3-4 weeks, when plantlets had 4-5 cm, was added 30 ml tuberisation liquid medium (Murashige-Skoog with 80 g/l sucrose, kinetin and coumarin). The experiment was made on laminar flux hood with sterile air.

For 18-20 weeks the culture was placed on darkness at 18-20ºC. The microtubers were harvested, washed, treated with fungicide, counted and sizing: <5mm; 5-10mm; >10mm.

Microtubers stored 60-80 days at 4ºC were passed in light conditions at 20±2ºC for sprouting, to be ready for planting in green house or in insect-proof tunnels. Microtubers smaller then 5 mm were inoculated again on regeneration and growing medium Murashige-Skoog to obtain microplants.

Observations were made on all material obtained from 320 jars with Christian variety and 281 jars with Roclas variety. On the base of microtubers, were done individual mean weight determination, weighing, microtuber by microtuber and than mediating/jar.

Results and discussion

From observations it was determined that Christian and Roclas Romanian varieties with a high capacity of field production (with middle size tubers), had in vitro a different behaviour.

There were significant differences between these varieties concerning the size, number and weight of microtubers. Christian variety produces more microtubers (2447) towards Roclas (1908) variety. Also the number of microtubers bigger than 5 mm, used for planting was higher (1692), comparatively with Roclas variety (697).

Varieties were different concerning microtubers size structure. The influence of medium conditions of potato for these varieties is presented in Figure 1.

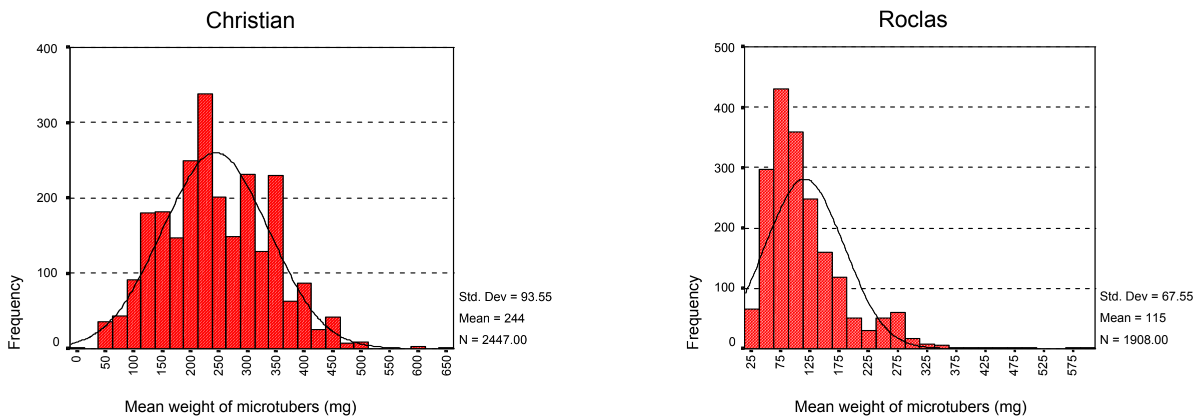

Figure 1. Histograms of microtubers mean weight at Christian and Roclas varieties

This histograms presents the difference previous presented, regarding the frequencies of microtubers individual weight. For Christian variety the mean weight of microtubers is 244 mg, while for Roclas variety is 115 mg.

Histograms of this two varieties in which are presented relative frequencies of microtubers weight, illustrate the difference anterior determined.

The strong assimetry at Roclas variety, is owing to higher frequencies of small microtubers and sporadically frequencies of large microtubers (Figure 1).

The average values and statistical properties of studied variable are presented on Table 1. From date analyses it has been ascertained that the capacity of Christian variety microtuber production was higher comparatively with Roclas variety, through the higher number of microtubers produced/jar and higher weight of this also.

Table 1.Statistical properties of microtubers obtained from Christian and Roclas varieties

Variabile	*UM*	**Christian**				**Roclas**			
		Mean	**Standard deviation**	**Standard error mean**	**CV%**	**Mean**	**Standard deviation**	**Standard error mean**	**CV %**
Number of microtubers:									
<5mm	no/jar	2.3	1.5	0.1	65.2	3.8	1.8	0.2	47.4
5-10mm	no/jar	3.5	1.8	0.1	50.0	2.4	1.8	0.2	75.0
>10 mm	no/jar	2.7	1. 5	0.1	55.6	1.5	1.3	0.2	86.7
>5 mm	no/jar	6.1	2.2	0.2	35.1	4.0	2.2	0.3	55.0
Percent >5 mm	%	72.0	15.2	1.2	21.1	49.0	26.6	3.4	54.3
Total number	**no/jar**	**8.5**	**2.3**	**0.2**	**27.1**	**7.7**	**2.4**	**0.3**	**31.2**
Mean weight									
<5mm	mg/jar	154.7	35.8	2.8	23.1	91.3	39.1	5.0	42.8
5-10mm	mg/jar	244.7	31.7	2.4	13.0	160.5	47.4	6.0	29.5
>10 mm	mg/jar	350.4	42.0	3.2	12.0	216.7	68.4	8.7	31.6
Mean	mg/jar	**253.6**	**35.3**	**2.7**	**14.0**	**133.3**	**45.1**	**5.8**	**33.8**

Except microtubers smaller than 5 mm, microtubers from Christian variety presents a high uniformity from jar to jar, than those of Roclas variety.

Christian variety present variation coefficients for medium number of microtuber/jar equal with 27.1%, while at Roclas is 31.2%. Also, variation coefficients for mean weight of microtubers at Christian variety was 14%, while at Roclas was 33.8%. Determined differences for this two studied varieties appeared stronger on examination the microtubers size fractions (Table 2).

Table 2. The influence of varieties on microtuberisation

Variable	**UM**	**Mean**		**Mean difference**	**Standard error mean**	**Significance**
		Christian	**Roclas**			
Number of microtubers:						
<5mm	no/jar	2.3	3.8	-1.5	36.8	.000
5-10mm	no/jar	3.5	2.4	1.1	15.2	.000
>10 mm	no/jar	2.7	1.5	1.2	30.6	.000
>5 mm	no/jar	6.1	4.0	2.1	43.6	.000
Percent >5mm	%	72.0	49.0	23.0	66.2	.000
Total number	**no/jar**	**8.5**	**7.7**	**0.8**	**5.3**	**.023**
Mean weight						
<5mm	mg/jar	154.7	91.3	63.4	135.4	.000
5-10mm	mg/jar	244.7	160.5	84.2	240.7	.000
>10 mm	mg/jar	350.4	216.7	133.7	321.3	.000
Mean	mg/jar	**253.6**	**133.3**	**120.3**	**450.6**	**.000**

Liberty degrees= 228

Varieties influence on microtuberisation was studied comparatively using variance analyze for both varieties, taking into account the number of microtubers on size fractions and mean weight of this.

Mean differences tested with standard error, indicate that Christian variety has a stronger microtuberisation than Roclas variety, results which concur with other observations.

Christian variety produce an average 8.5 microtubers/jar, exceed Roclas variety with 0.8 microtubers/jar.

On this variety the number of microtuber > than 5 mm, useful directly in propagation process was 6.1 microtubers/jar (72% from total) comparatively with 4.0 microtubers/jar obtained from Roclas variety (49.0% from total).

The highest weight of microtubers from all fractions at Christian variety, an average 253.6 mg/jar, towards 133,3 mg/jar, indicate an increased vigour on propagation process (Table 2).

Conclusions

- the experimental results concerning microtubers production proved that Roclas and Christian varieties had different genetic capacity of in vitro plantlets tuberisation.
- on the same condition of tuberisation Christian variety produce more microtubers with a weight mean higher, comparatively with Roclas variety.

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RELATIONSHIP BETWEEN MICROTUBER YIELD AND THE RATE OF PERIMEDULLA UNDER DIFFERENT TUBERIZATION CONDITIONS

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Abstract

The effects of tuberization conditions on the production capacities of large sized microtubers (8-16 mm) and the proportion of perimedullary region were studied. *In vitro* tuberization was induced on explants with different nodes layered on hormone-free MS medium. For volume calculations of different tissue regions, the formula for an ellipsoid (V=4/3π1/8lw^2) was used. The microtuber size was affected by sucrose support and explant type. Microtubers have well-developed perimedullary region, and its volume rate seemed to be important in the final size of tubers because its increase was connected to the increase of tuber size until tubers reached 12 mm diameter.

Keywords: *in vitro*, tuber volume, tuber size, perimedulla

Introduction

The economical use of *in vitro* tubers is mainly depends on their size because larger microtubers have greater early vigour, emergence and performance and they are able to produce larger crop than small ones (13,19,22). The size of microtubers can be increased by applying an adequate photoperiod regime (2,14), culture density (4,20) type of explants (7,11) or proper nitrogen and sucrose concentrations in the medium (1,5,12,15,16) and so on. As a results of above-mentioned manipulations, a part of microtubers developed was larger sized but their final size seldom or no exceeded 10 mm (1,14,15,18,21).

After stolon swelling the tuber growth continues, especially in the perimedullary region of tubers. However, this tissue region does not or slightly develop in *in vitro* grown tubers and this may be hypothesized to limit the final size of *in vitro* tubers around 10 mm. (17). Nevertheless, earlier we have produced *in vitro* tubers on hormone-free medium, from which 11-29% of microtubers were produced with larger than 10 mm in diameter up to 16 mm (9) in cv. Desiree.

The aims of present work include the investigation of production facilities of large sized microtubers in other potato varieties (cv. Boró, cv. Gülbaba) and the study of the effects of the applied tuberization conditions on the proportion of microtuber tissues, especially on the perimedullary region.

Materials and Methods

Tuberization was induced on fully developed potato plantlets by pouring of 8 % sucrose solution onto the cultures as described earlier (3) in the control treatment. Explants with 2 or 5 nodes were layered on a medium containing MS salts and vitamins (10) supplemented with 8 % sucrose and 0.8 % agar-agar in the other two treatments: 15 or 6 explants per jar were cultured containing 2 or 5 nodes, respectively, thus the total number of nodes per jar were the same (30 nodes) for each treatment. Induced cultures were exposed to short days (8 h) for 2 weeks, then to total darkness for further 11 weeks (3).

At the end of experiments microtubers were harvested and graded by their smallest diameter, and the number of tubers per jar, their size distribution, their fresh weight and the multiplication rate defined as number of microtubers per explant were recorded. Fifteen jars were observed in each treatment. Ten microtubers from each size fractions in every treatment were cut in half longitudinal and they were used for calculation of volume of fresh tubers and their tissues according to the method of Liu and Xie (2001), which based on the formula for an ellipsoid: V=4/3π1/8lw^2= 0.52lw^2, where l: length of tuber, w_1: width of pith tissue, w_2: total width of the perimedulla and pith tissues, w_3: total width of the cortex, perimedulla and pith tissues. Calculations of the volumes: cortex: V_{co}= 0.52$l(w_3^2-w_2^2)$, perimedulla: V_{pe}= 0.52$l(w_2^2-w_1^2)$, pith: V_{pi}= 0.52lw_1^2. Experiments were repeated three times. Data were analysed by ANOVA followed by Tukey' test using SPSS 7.5 for *Windows* programme.

Results and Discussion

The multiplication rate (MR) was significantly influenced by treatments and varieties (Table 1). If explants with 2 nodes were layered on tuberization medium, the MR decreased with 10-22% in Desiree and Gülbaba but it increased with 26% in Boró compared to the control treatment. The highest MR was obtained for explants with 5 nodes in each cultivar.

Table 1. Effect of different tuberization treatments on the multiplication rate, average fresh weight of microtubers, on the total number of microtubers per jar and on the number of large sized microtubers per jar*

Cultivar	**Treatments**	**Multiplication rate**	**Average fresh weight of tubers (mg)**	**Tuber number per jar**	
				Total	**> 8 mm**
Desiree	Control	1.35 b	1.35 b	36.5 c	0.5
	Explant with 2 nodes	1.06 a	199 b	15.9 b	8.5
	Explant with 5 nodes	1.62 c	368 c	9.7 a	5.2
Gülbaba	Control	1.46 b	68 a	43.8 c	0.9
	Explant with 2 nodes	1.32 a	294 b	19.7 b	10.8
	Explant with 5 nodes	1.88 c	493 c	11.3 a	6.7
Boró	Control	1.06 a	82 a	31.7 c	0.6
	Explant with 2 nodes	1.34 b	208 b	20.1 b	7.6
	Explant with 5 nodes	1.89 c	278 c	12 a	5.3

Average fresh weight of microtubers (AFW) increased significantly compared to the control treatment in each cultivar. If explants with 2 nodes were cultured on tuberization medium, increase of AFW was 2.5-4.3-fold but in the case of explants with 5 nodes it was 3.4-7.2-fold depending on cultivars. The total number of microtubers per jar (TNT) was always the highest in the control treatment and it decreased with the increase of size of the explants. The number of tubers larger than 8 mm per jar (NLT) was only 2% in the control treatment. However, in the other two treatments NLT increased up to 38-59% depending on cultivars and on the treatments.

Concerning the sizes of tissue regions measured and their calculated volumes, data indicate significant effects of treatments and tuber size on the volumes of tubers and on the volumes of the different tissue regions. The correlations between the volume rate of different tissue regions or whole volume of tubers and tuber size are presented in Table 2.

In correlation analyses between the volume rate of different tuber tissues and tuber size only tubers sized between 4-12 mm were considered because under 4 mm tuber diameter the volume rate of the cortex but above 12 mm tuber diameter the volume rate of the pith were too high, which would have distorted the correlation. The volume of tubers is related significantly to tuber size by power function in every treatment in each cultivar at $p < 0.01$. The value of allometric exponent *b* was with 19-45% higher if explants with 2 nodes were tuberized and with 14-80% higher if explants with 5 nodes were tuberized than in the control treatment.

In the case of cv. Desiree the volume rate of cortex region (V_{co}/V) varied between 37-44% in the control treatment but it decreased with the increase of the tuber size (explants with 2 nodes: from 35% to 25% or explants with 5 nodes: from 37% to 20%). However, no any significant correlation could be detected. The volume rate of perimedulla (V_{pe}/V) varied between 34-55% depending on tuberization treatments but no any significant correlation could be obtained between it and the tubers size. If the size of tubers was larger than 4 mm, the volume rate of the pith (V_{pi}/V) appreciably depended on the tuberization treatment (*in control treatment*: 14-22%; *explants with 2 nodes*: it decreased from 23% to 16% till tubers reached 10 mm; tubers larger than 10 mm it was 22% again; *explants with 5 nodes*: it increase with increase of tuber size from 18% up to 41% and this correlation was proved to be significant at $p < 0.01$). V_{co}/V showed decreasing tendency if the size of tubers increased in all of the treatments in cv. Boró but the correlation was not significant. V_{pe}/V varied between 33-43% and no any important relationship with the tuber size could be detected. However, V_{pi}/V increased with the increase of tuber size from 16% to 22% in the control treatment, from 15% to 33% if explants with 2 nodes were tuberized and from 20% up to 32% if explants with 5 nodes were tuberized and these correlations were proved to be statistically significant at $p < 0.01$. V_{co}/V decreased but V_{pe}/V and V_{pi}/V increased significantly with increase of tuber size in all of the treatment in cv. Gülbaba, except V_{pi}/V when explants with 5 nodes were tuberized.

Statistical analysis proved significant correlation between V_{co}/V and V_{pe}/V in every cultivar and treatment; moreover, correlation between V_{pe}/V and V_{pi}/V was significant in cv. Desiree in every treatment and in cv. Gülbaba in the control treatment (Tab. 2).

Table 2. Effects of tuberization treatments on the correlations between the parameters of microtubers. (Abbreviations: V_{co}/V: volume rate of the cortex, V_{pe}/V: volume rate of the perimedulla, V_{pi}/V: volume rate of the pith, F: tuber size, V: volume of the microtuber, n.s.: non significant, **: significant at $p < 0.05$, ***: significant at $p < 0.01$)

Correlations	Tuberization treatment	Desiree	Boró	Gülbaba
V - F	control treatment	$V=22.40\ F^{1.92}$ $r^2=0.904^{***}$	$V=24.72\ F^{1.54}$ $r^2=0.873^{***}$	$V=25.73\ F^{1.41}$ $r^2=0.823^{***}$
	explant with 2 nodes	$V=13.86\ F^{2.29}$ $r^2=0.932^{***}$	$V=19.73\ F^{2.07}$ $r^2=0.946^{***}$	$V=19.74\ F^{2.05}$ $r^2=0.945^{***}$
	explant with 5 nodes	$V=17.86\ F^{2.19}$ $r^2=0.889^{***}$	$V=9.84\ F^{2.58}$ $r^2=0.948^{***}$	$V=10.49\ F^{2.54}$ $r^2=0.976^{***}$
V_{co}/V - F	control treatment	*n.s.*	*n.s.*	$V_{co}/V= 65.18\ F^{-0.69}$ ***
V_{pe}/V - F		*n.s.*	*n.s.*	$V_{pe}/V= 18.30\ F^{0.79}$ ***
V_{pi}/V - F		*n.s.*	$V_{pi}/V= 10.71\ F^{0.62}$ ***	$V_{pi}/V= 9.79\ F^{0.96}$ ***
V_{co}/V - V_{pe}/V	4mm<F<12mm	$V_{pe}/V=-0.88\ V_{co}/V + 78.18$ ***	$V_{pe}/V=-0.63\ V_{co}/V + 65.21$ ***	$V_{pe}/V=-0.61\ V_{co}/V + 61.27$ ***
V_{pi}/V - V_{pe}/V		$V_{pe}/V=-0.80\ V_{pi}/V + 56.16$ ***	*n.s.*	$V_{pe}/V= 0.51\ V_{pi}/V + 6.76$ ***
V_{co}/V - F	explant with 2 nodes	*n.s.*	*n.s.*	$V_{co}/V= 69.38\ F^{-0.70}$ ***
V_{pe}/V - F		*n.s.*	*n.s.*	$V_{pe}/V= 21.45\ F^{0.47}$ ***
V_{pi}/V - F		*n.s.*	$V_{pi}/V= 13.78\ F^{0.50}$ ***	$V_{pi}/V= 9.73\ F^{0.80}$ ***
V_{co}/V - V_{pe}/V	4mm<F<12mm	$V_{pe}/V=-0.91\ V_{co}/V + 77.92$ ***	$V_{pe}/V=-0.36\ V_{co}/V + 49.05$ ***	$V_{pe}/V=-0.56\ V_{co}/V + 58.70$ ***
V_{pi}/V - V_{pe}/V		$V_{pe}/V=-0.90\ V_{pi}/V + 66.42$ ***	*n.s.*	*n.s.*
V_{co}/V - F	explant with 5 nodes	*n.s.*	*n.s.*	$V_{co}/V= 60.80\ F^{-0.27}$ ***
V_{pe}/V - F		*n.s.*	*n.s.*	$V_{pe}/V= 25.25\ F^{0.24}$ ***
V_{pi}/V - F		$V_{pi}/V= 17.29\ F^{0.32}$ **	$V_{pi}/V= 17.30\ F^{0.34}$ ***	*n.s.*
V_{co}/V - V_{pe}/V	4mm<F<12mm	$V_{pe}/V=-0.70\ V_{co}/V + 64.33$ ***	$V_{pe}/V=-0.48\ V_{co}/V + 55.57$ ***	$V_{pe}/V=-0.69\ V_{co}/V + 67.05$ ***
V_{pi}/V - V_{pe}/V		$V_{pe}/V=-0.55\ V_{pi}/V + 57.96$ **	*n.s.*	*n.s.*

In the experiments the production of microtubers occurred on hormone-free medium by different way of sucrose support and different types of explants. It can be concluded, that the size of microtubers could be increase by appropriate way of sucrose support and explant type. Number of large sized tubers (> 8 mm, up to 16 mm) reached 44% to 59% depending on cultivars. If explants with 2 nodes were cultured on tuberization medium, both AFW (199 to 294 mg) and NLT (38% to 55%) were high enough besides satisfying TNT (15.9 to 20.1) as in Table 1.

Our results suggest, that microtubers produced on hormone-free medium have well-developed perimedullary region, and its volume rate seemed to be important in the final size of tubers. Correlation analysis proved that with the decrease of the volume rates of the cortex and perimedullary regions (V_{co}/V, V_{pe}/V) increased. Increase of V_{pe}/V was connected to the increase of tuber size until tubers reached 12 mm diameter. Between V_{pe}/V and tuber size direct relationship was detected in cv. Gülbaba, but it was indirect in the other two cultivars (Table 2). It could be supposed, that, as in the case of field-grown tubers, the increase of V_{pe}/V could be one of the important factors influencing the capacity of microtubers to act as sink for assimilates. It could be particularly true if the increase of V_{pe}/V is connected with the increase of the cell number, which was not examined in these experiments, but was proved by others under *in vitro* conditions (8). However, in microtubers larger than 12 mm the V_{pe}/V did not increase anymore and the maximal tuber size was 16 mm (w_3). It seems, that this is the maximum tuber size, which could be reached on hormone-free medium, if we would like to obtain also economically sufficient MR and TNT in a jar.

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EFFECT OF MANNITOL-INDUCED OSMOTIC STRESS ON THE CONTENT OF CARBOHYDRATE IN POTATO CALLI

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One of the most important abiotic stresses is the shortage of water to plants. Potato is a drought-susceptible crop, however, there are differences in the susceptibiliy to water stress between cultivars.

Three potato clones with different drought tolerance were examined as in callus-tests ('Boró', 'Réka', 736/82). Calli were grown on 0.8 M mannitol added to MS medium. Among the changes we tested the three main types of sugars (fructose, glucose, sucrose).

Among potato clones have already detected differences in homeostatic. Mannitol-induced abiotic stress caused higher differences in the carbohydrate content between cultivars, than in control treatment.

EFFECT OF BIOTIC STRESS ON THE ACTIVITY OF STRESS ENZYMES IN POTATO PLANTLETS

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Potatoes are affected by many bacteria. One of the more important diseases is soft rot, which affects both stems and tubers. In the experiments the virulent strains of the *Erwinia carotovora* subs. *carotovora* were used.

Three potato clones with different resistance against *Erwinia* ssp. were used as test plants: 77365/103 (resistant), 'Réka' (moderate susceptible) and 98/91 (susceptible). The shoots were propagated on hormone-free MS medium.

We have studied the relationship between biotic stress tolerance of different potato clones and some biochemical properties: the activity of the enzymes peroxidase (POD) and polyphenol oxidase (PPO).

EFFECT OF HAULM APPLICATION OF GIBBERELLIC ACID ON THE PRODUCTION AND DORMANCY OF MINITUBERS OF POTATO CV SPUNTA

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Summary

Miniplants produced by microparopagation were cultivated in pots and treated once with GA_3 (10, 50 or 100 ppm) at 10, 20 or 30 days before lifting. Control plants received no treatment. Plant height was not affected by either the concentration of GA_3 or the time of application and no differences in the weight of tubers per plant were observed. The application of GA_3 resulted in dormancy breakage and the formation of sprouts on the new tubers, but secondary tubers were formed at higher concentrations.

Introduction

At harvest, potato (*Solanum tuberosum* L) tubers are normally dormant. For potato growers in countries with more than one planting season it may be desirable to break tuber dormancy rapidly, and search for ways to shorten potato tuber dormancy has been the subject of considerable work. For example, multiple chemical methods have been tested, but with mixed success (Denny, 1926; Burton 1989), and dormancy reduction by gibberellic acid (GAs) is well documented. When cut potato tubers are dipped or soaked in GA, the length of dormancy is shortened, but abnormally elongated sprouts and morphological deviations may arise (Rappaport *et al*., 1957; Timm *et al*., 1960; Choudhuri & Ghose, 1963). Van Ittersum & Scholte (1993) performed haulm applications of GA on potato plants and achieved a reduction in length of dormancy of seed potatoes by several weeks. Apart from its effect on dormancy, haulm application of GA increases leaf area, dry matter and the dry weight of tubers (Humphries, 1958).

Materials and Methods

Presprouted potato tubers of the cv Spunta, which were under thermotherapy for fourty days, at 36 °C and 95% humidity, were used as mother propagating material. Plantlets were produced *in vitro* on a modified MS medium, supplemented with 3% sucrose, 4.5 mg/l BAP, O.009 mg/l IBA, 55.7 mg/l ascorbic acid and solidified with 2.5 g/l phytagel (Gregoriou *et al*., 2007). They were hardened in transplanting trays of 77 small cells (4x4x7cm, w,l,d) filled with potting soil.

Miniplants from micropropagation were placed in growth chambers (20/10 °C, day/night temperature with 16h light, 80% RH and illumination of 20000 lux for 16 hours in a SANYO MLR 351 H growth chamber) on 13/12/2006 and planted in pots under a net-covered cage (nethouse) at the Agricultural Research Institute in Nicosia on 27/12/2006. Planting was done in a mixture of two parts of potting soil and one part of vermiculite in pots of 18 cm diameter. Drippers of 2 l/h capacity on 16 mm diameter polyethylene line were used for irrigation. Fertilizers were applied through a fertigation system with a Dosadron D300 fertilizer injector at the rates of 70 ppm N, 20 ppm P and 100 ppm K, including micronutrients. Three treatments were applied by spraying the plants with 10, 50 or 100 ppm gibberellic acid (GA_3, Valent Biosciences Corporation, USA) at 30, 20 and 10 days before lifting (DBL), corresponding to 13/3/2007, 23/3/2007 and 2/4/2007, respectively. The control plants received no treatment. Tubers were lifted on 12 and 13/4/2007.

The experimental plan was a Randomized Complete Block Design (RCBD) with four replications. Each block included sixteen pots from which the four pots in the middle were used for acquisition of data. Records were kept of the number and weight of tubers, tuber distribution, plant height, branching within the canopy, the number of sprouts per tubers and the formation of secondary or sprout tubers.

Pre-sprouted potato tubers were used as mother propagating material and the plants produced by *in vitro* culture were planted in the nethouse and indexed by ELISA and macroscopic examination for the potato leaf roll virus (PLRV), potato virus Y (PVY), potato virus A (PVA), potato virus S (PVS), potato virus X (PVX) and potato virus M (PVM).

Randomly selected samples of the harvested tubers were sent to the Laboratory of Vegetable Production of the Agricultural University of Athens to be examined for dormancy breakage and sprouting. These tubers were planted singly in pots on 21/4/2007 with four replicates of eight tubers per treatment. The emergence and number of sprouts per tuber were recorded.

Results

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Haulm applications of GA_3 10 or 20 days before harvest had no effect, but GA_3 treatment 30 days before harvest resulted in a statistically significant increase in the number of tubers per plant (Table 1). Mean tuber weight declined as the time from spraying to harvest increased.

Neither the concentration of GA_3 nor the time of application had a statistically significant effect on the weight of tubers per plant. The weight of tubers per pot was 149, 157, 144 and 145 g for the control, 10, 50 and 100 ppm GA_3, respectively, and 152, 146 and 147g for the 1st, 2nd and 3rd time of application, respectively.

Table 1: Number of tubers per plant in relation to haulm applications of GA_3 10, 20 and 30 days before lifting.

GA_3 ppm	**Time (days before harvest)**					
	Number of tubers			**Mean tuber weight (g)**		
	10	20	30	10	20	30
0 (Control)	7.3a[1(ns)]	7.1a[(ns)]	7.9a(b)	22.6a[(ns)]	21.9a[(ns)]	19.9a(a)
10	7.6b	8.8ab	10.6a(ab)	22.6a	18.5b	16.6b(ab)
50	6.3b	8.5b	12.9a(ab)	24.1a	19.1a	13.1b(b)
100	7.8b	8.1b	11.9a(a)	20.9a	18.4a	13.3b(b)

[1] The same letter in a row for each GA treatment indicates no significant difference between the spray periods as determined by an LSD test at 5% level. The same letter in brackets in a column indicates no significant differences between the spray treatments.
[ns] non significant

The tuber size distribution for the treatments applied 10 or 20 DBL was similar with about 60% of the tubers being larger than 10g (Table 2). By contrast, the percentage of tubers <5g following GA_3 treatment at 50 or 100 ppm 30 DBL was almost 55% of the total number of tubers.

Plant height was not affected by either the concentration of GA_3 or the time of application. Plant height was 21.2, 20.9, 20.8 and 23.2 cm for the control, 10, 50 and 100ppm GA_3, respectively. The application of GA_3 resulted in the development of new branches within the canopy of the plant. The number of new branches per plant was approximately three for all the GA_3 concentrations. The number of branches rose as the time between treatments and harvest increased (0.9, 2.5 and 3.6 for 10, 20 and 30 days, respectively).

The application of GA_3 not only affected the breakage of dormancy, but also resulted in the formation of secondary tubers. The number of sprouts on the new tubers increased with increasing GA_3 concentration. No sprouts formed on the tubers of the control. The average number of sprouts per tuber was 1.8, 3.1 and 3.9 for 10, 50 and 100 ppm GA_3 respectively. The formation of new tubers ranged form 1.7 to 2.3 for the three GA_3 concentrations, but was not statistically significant. No secondary tubers were found on tubers derived from plants that had been treated 10 DBL.

Table 2: Weight distribution by class and mean tuber weight in relation to GA_3 treatments 10, 20 and 30 days before lifting.

GA_3 ppm	Time (days)	Weight distribution by class (%)			
		< 5 g	5-10 g	>10-20 g	> 20 g
0 (Control)	10	19	17	23	41
10	10	16	19	14	51
50	10	16	16	15	53
100	10	21	15	21	43
0 (Control)	20	20	12	19	49
10	20	30	14	14	42
50	20	25	9	19	47
100	20	22	17	23	38
0 (Control)	30	23	13	27	37
10	30	27	18	22	33
50	30	39	14	23	24
100	30	43	12	16	29

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The time to dormancy breakage and the number of sprouts per tuber was related to both the concentration of GA_3 and the time of application prior to harvest GA_3 (Fig. 1). Tubers sprouted quickest and to a high percentage following the application of 100 ppm GA_3 30 DBL and to the same percent, but approximately 2 weeks slower when the same concentration was applied 10 DBL.

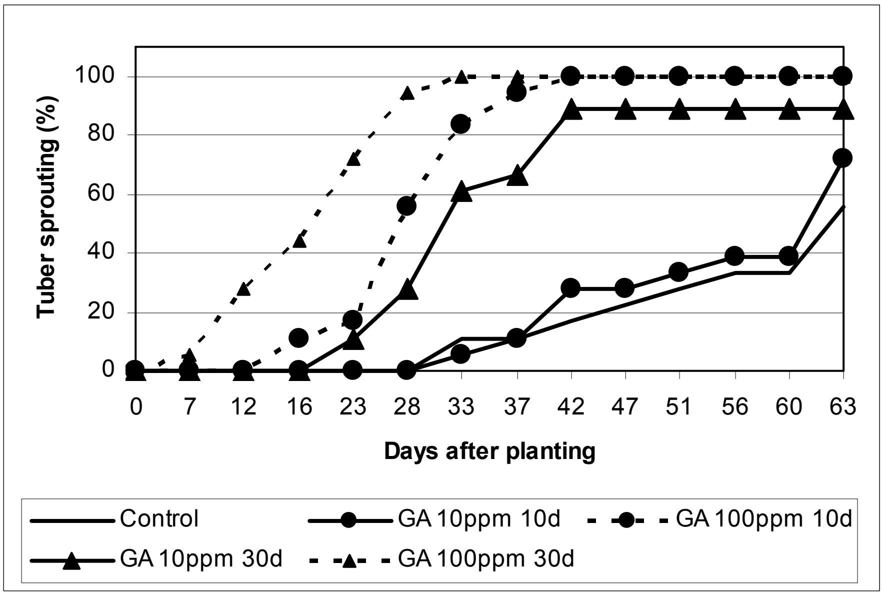

Figure 1: Time course of the sprouting of potato tubers produced by plants derived from minitubers in relation to GA_3 application 10 or 30 days before lifting.

The lowest concentration of GA_3 (10 ppm) also promoted sprouting, but to a lower percent than the 100 ppm treatment, when applied 30 DBL, but the same concentration of GA_3 applied 10 DBL had no effect on sprouting.

From the present experiments it is concluded that the length of dormancy of seed tubers produced by plants derived from minitubers may be reduced by the haulm application of GA_3 prior to harvest. The optimum application is 100 ppm GA_3 applied 30 DBL. This treatment not only increases the number of tubers formed per plant, but also results to the shortest sprouting time.

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THE EFFECT OF PHYSIOLOGICAL AGE AND GROWTH REGULATORS ON MICROTUBERS DORMANCY AND VIGOUR

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Introduction: In the last years the interest in potato multiplication by microtubers considerably increased. Their implementation into the breeding practice allows to halve the time of seed potato production. Heretofore microtubers are treated as means of potato multiplication alternative to plant propagation *in vitro*; however, due to possibility of their production all year round, it is much more effective. The most important problem is the adaptation of physiological age of seed material to the date of planting and shortening or breaking dormancy period. However, the duration of this period is a genetic trait independent on the length of vegetation of a particular cultivar. In potato seed production, dormancy period was hitherto broken by use of the routine methods for the tuber indexing, where not whole tubers, which are very resistant to this treatment, were used, but only their pieces. Actually, an information is missing concerning possibilities of shortening dormancy period of microtubers in different physiological age. Another problem is the vigour of microtubers – whether it is sufficiently high for effective potato minituber production. In this study, the vigour of microtubers was evaluated using the multiplication coefficient.

Material and methods. In 2007, the experiment was carried out with four cultivars: Irys (very early), Adam, Cekin, Irga (medium-early). As growth regulators thiourea and gibberellin were used in two doses: 100 or 200% of the standard concentrations which are normally applied in tuber indexing. The treatment with distilled water served as control. This procedure was executed on the 7th of May, with physiologically very young microtubers, obtained from *in vitro* in March. At planting they were in the state of physiological dormancy. The microtubers were planted on the 15th of May and minitubers were harvested on the 20th of August 2007.

Results. Significant effect of the applied growth regulators on number of sprouted microtubers was confirmed. The effect of the 100% concentration consisted in 70.0% of sprouted microtubers and the effect of 200% concentration – in 96.5% of sprouting. However, the reaction of particular cultivars was different. For the cultivar Irys, characterised by a short dormancy period, 100% concentration was sufficient, whereas for the slowly ageing cultivars, Adam, Cekin and Irga, the 200% concentration of growth regulators was more effective. In general, the effect of applied growth regulators on the multiplication coefficient of microtubers was positive. The reaction of particular cultivars was, however, different. The physiologically slowly ageing cultivars Adam, Cekin and Irga, increased their coefficient of multiplication in response to the higher concentration of growth regulators (by 77%), whereas the quickly ageing 'Irys' showed an opposite reaction – a gradual decrease of the tested coefficient. Growth regulators also influenced significantly the size of minitubers.

Conclusion. The tested growth regulators may effectively break the dormancy period of potato microtubers; however their concentration should be adapted to their physiological age. Thiourea and gibberellin, used in the tested concentrations, may increase the multiplication coefficient of physiologically very young microtubers. However, at assessment of the usefulness of their application and at the choice of optimal concentration the rate of physiological ageing of particular cultivars must be considered.

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IN VITRO CALCIUM DEFICIENCY OF *SOLANUM TUBEROSUM* CV. SANTÉ

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Abstract

Calcium is essential for plant nutrition, with an important structural, physiological and biochemical role for the cell. The goal of this study is the identification and elimination of *in vitro* calcium deficiency of *Solanum tuberosum* cultivar Santé. In this experiment we used plantlets with calcium deficiency symptoms: curled leaves, with necrotic areas, lateral branching, poor root growth and growth ceasing. The cuttings were inoculated on Murashige-Skoog growth media modified with calcium pantothenate, in three variants: M0 – the control sample (no calcium added), M1 with 200 mg/l and M2 with 400 mg/l calcium pantothenate. The explants were kept to 16 h light/ 8 h dark, at 21, 6°C, in the growth room.

Keywords: calcium pantothenate, deficiency, cv. Santé

Introduction

Calcium is an essential macronutrient for the animal cell but also for the vegetal one. It is involved in the cellular processes which maintain the structural and functional integrity of cellular membrane, stabilizing the structure of cellular wall, involved in ion transport and controlling the ion exchange, also controlling the cell wall enzymatic activity (Grattan and Grieve, 1999). Another important role is in activation of enzyme calmodulin, responsible for the initiation of a series of physiological answers in plant, like geotropism (Nilsen and Orcutt, 1996). Calcium ions and calmodulin are also implicated in cell multiplication control in eukaryotes, including plants. Studies show that cells need a millimolar quantity of extracellular calcium to proliferate. Also, the cellular cycle of a normal cell is associated with rapid changes in intracellular concentration of calcium. Manipulating cytosolic concentrations of calcium proved to affect the events from cell cycle. Rapid changes in calcium concentration are involved in quiescence, G_2/M transition, mitosis final stage and G_1/S transition. Events of mitosis like nuclear membrane disintegration, chromatin condensation and anaphase beginning are all correlated with rapid growth of intracellular calcium level. More, these events can be premature induced by artificial growth of intracellular calcium level (Reddy and Day, 2002).

High level of calcium determines the growth of tolerance level to salinity in plants. The effect is mediated through the growth of intracellular level of calcium, vacuolar pH changes and activation of vacuolar Na/H antiporter. Strict control upon the cytosolic calcium level and calcium storage in different locations (vacuoles, mitochondria, endoplasmic reticulum) proves the crucial role of calcium in salinity stress response of plants (Bohnert and Cushman, 2002).

On the other side, is essential for plants to control the calcium quantities dissolved in cell. The concentration must be maintained to low level; otherwise the calcium negatively affects the metabolism. Plant crystallizes the calcium to produce oxalic acid. Calcium and oxalic acid interact and form calcium oxalate, which is insoluble. Crystallized calcium is much more inert and doesn't interfere in plant's metabolism (Mauseth, 2003).

Many enzymes are calcium dependent, and calcium is co-factor in the enzymes responsible for ATP hydrolysis. As major cation, calcium helps creating a balance between anions within the plant, but unlike other cations, calcium isn't readily mobile (George, 1993). Also, calcium is in a sensible balance with magnesium and potassium ions, bigger quantities from one or another could cause deficiency in the other (Vitosh et. al., 1994).

Calcium deficiency is rare in nature, especially in acid soils (< pH 5.0) or when large quantities of potassium and magnesium are used. Even moderate acid soils (pH between 5.0 and 6.0) generally contain large quantities of calcium (Vitosh et. al., 1994), the ion availability being determinate by pH (Fig. 1). From all the plant organs, the roots are the most affected by soil or growth medium pH. H^+ excess in growth medium affects the plant growth in two ways: unspecific – by inhibiting roots growth, lateral branching and water absorption, and specific - hydrogen ions compete with other cations, being absorbed with preponderance (Alam et. al., 1999).

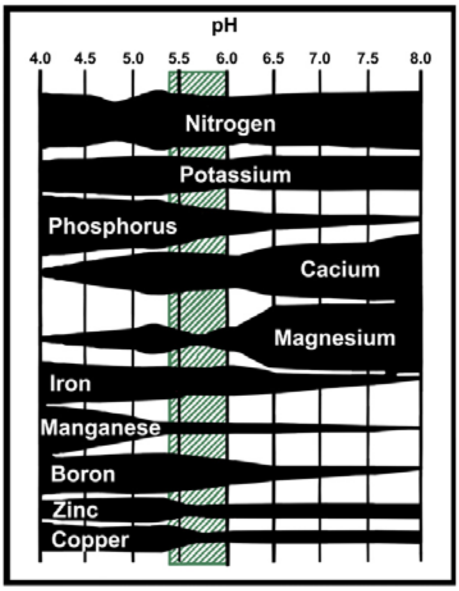

Figure1. Nutrients availability affected by pH. The black zones indicate the relative availability of elements (Pennisi and Thomas, 2005)

Calcium deficiency in plants consists in poor root growth, necrosis and curling of leaves, growth ceasing and terminal buds death. After terminal buds death, the plant is developing new lateral branches, and in extreme deficiency cases the terminal buds of lateral branches could die, developing other ones (George, 1993) (Fig. 2 - B).

Plants with calcium deficiency have a reduced meristematic activity as a result of the reduction of cellular division process and cellular expansion. This aspect is reflected in the deformed aspect and reduced size of leaves (Nilsen and Orcutt, 1996) (Fig. 2 - C).

Material and methods

For in vitro elimination of calcium deficiency of Solanum tuberosum cv. Santé, calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$) was added to Murashige-Skoog (MS) growth media. Calcium pantothenate was dissolved in double distilled water.

Calcium pantothenate is a pantothenic acid (vitamin B5) salt, a vitamin easily soluble in water, considerate an essential nutrient. It's been assumed that each vitamin from the B complex has a defined role in plant. Being produced by the plant, scientists assume that these vitamins have an important role in plant's life cycle even though there are no sufficient data to sustain this affirmation.

The experiment was done with three Murashige-Skoog variants:
M0 – MS growth media (control sample)
M1 – MS growth media with 200 mg/l calcium pantothenate
M2 – MS growth media with 400 mg/l calcium pantothenate

The test tubes used were sterilized in the hot air sterilizer, two hours at 180°C. After preparation, the growth media was poured in test tubes, covered with aluminum foil and sterilized in Raypa autoclave for 15 minutes at 121°C.

In vitro potato plantlets were multiplied using mini-cuttings method (single node with one leaf). The cuttings were inoculated on growth media, in three variants. After the inoculation, the cuttings were transferred in growth room, to 16 hours light/ 8 hours dark, at 21, 6°C.

After 3-4 weeks the regenerated plantlets were observed, morphological and statistical.

Results

After the first subculture the plantlets regenerated on MS growth media with calcium pantothenate developed well, the leaves are normal, without curled margins or necrotic tissue, roots very good developed (Fig. 3).

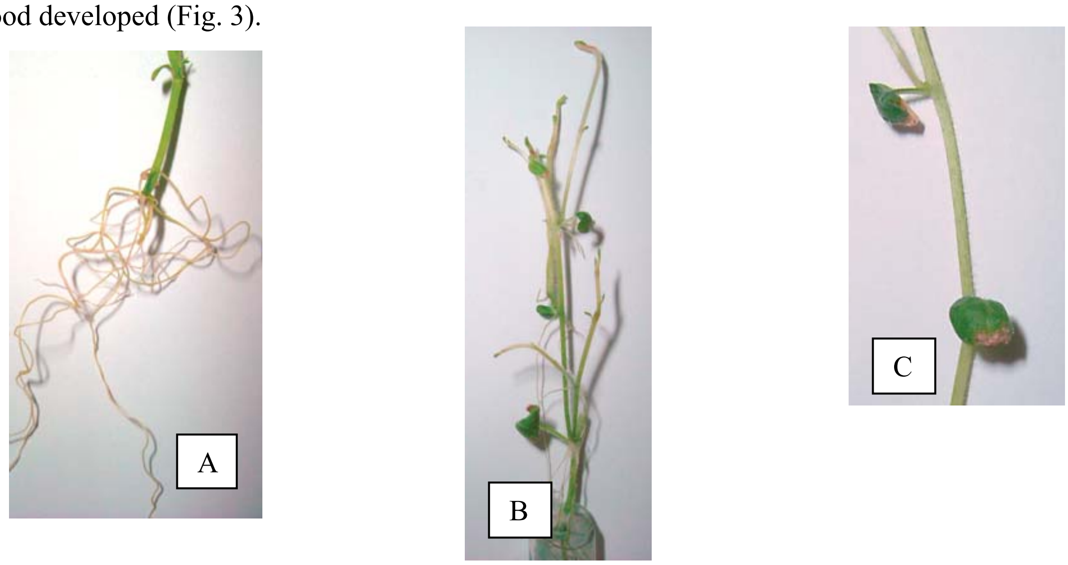

Figure 2. In vitro calcium deficiency of Solanum tuberosum cv. Santé
A – The root is very poor developed
B – Lateral branching phenomena associated with calcium deficiency
C – Curled leaves, with necrotic tissue

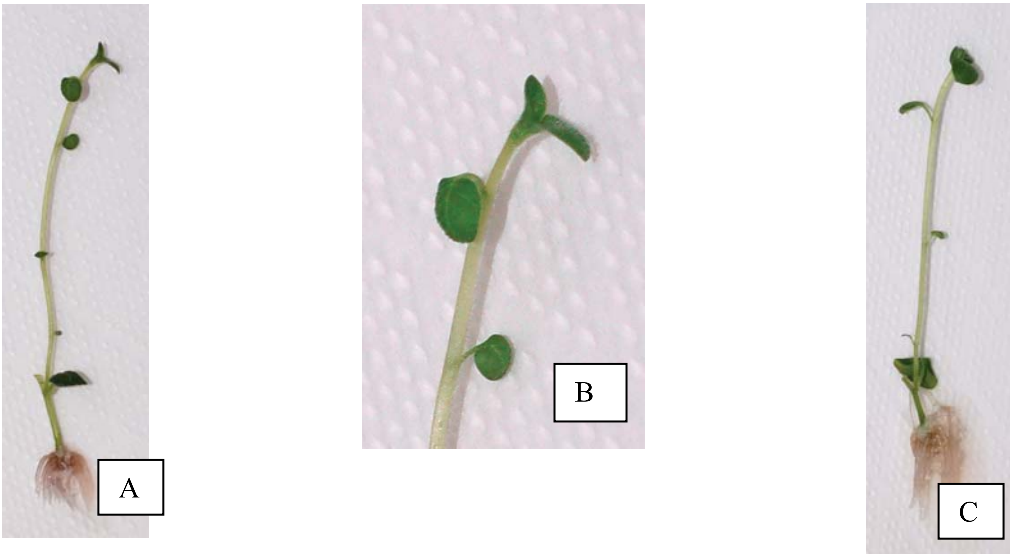

Figure3. Result obtained after adding calcium pantothenate to MS growth media
A – Regenerated plantlet on growth media M1 – general appearance
B – Regenerated plantlet on growth media M1 – leaves
C – Regenerated plantlet on growth media M2 – general appearance

The distance between two nodes is measured, to obtain an average for each growth media variant and subculture (Tab. 1, Fig. 4).

Analyzing the obtained data, we observed a reduced distance between two nodes for the plantlets grown on MS media with calcium pantothenate. Also, the regenerated plantlets are more uniform, after analyzing the standard deviation (Fig. 5).

Table1. MS growth media variants used in this study, average distance between two nodes and standard deviation for each variant and subculture.

Medium variants and subcultures	Average distance between two nodes (cm)	Standard deviation (cm)
M1 I	1.1	0.26
M2 I	1.09	0.26
M1 II	1.13	0.24
M2 II	1.03	0.35
M1 III	0.68	0.20
M2 III	0.64	0.25

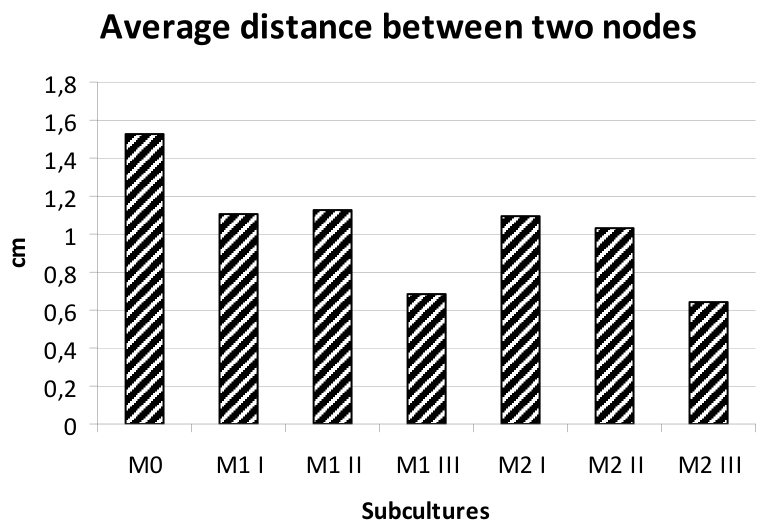

Figure4. MS growth media variants used and average distance between two nodes, calculated for each variant and subculture

M0 – control sample
M1 – MS medium with 200 mg/l calcium pantothenate
M2 – MS medium with 400 mg/l calcium pantothenate
M I – first subculture
M II – second subculture
M III – third subculture

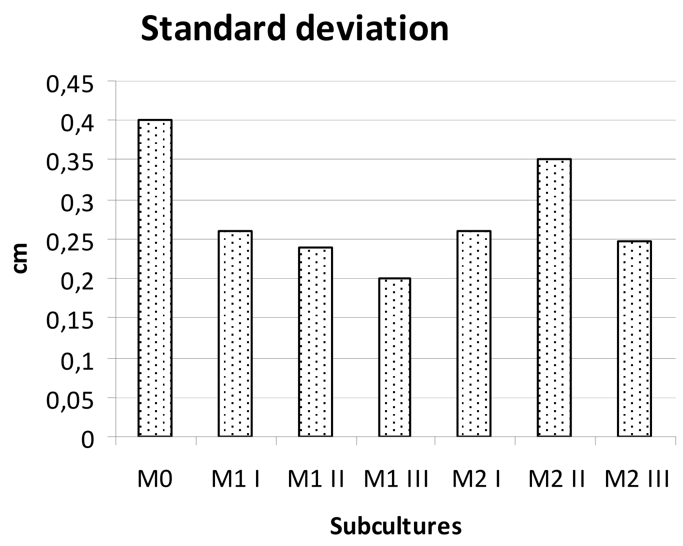

Figure5. MS growth media variants used and standard deviation calculated for each variant and subculture

After one subculture on growth media with calcium pantothenate, the regenerated plantlets were cut and inoculated on fresh MS growth media. The root was poorly developed (Fig. 7), normal leaves, but the distance between two nodes was significantly more reduced to subculture II (M1 and M2). Also the regenerated plantlets are more uniform, observations confirmed also by the standard deviation calculated for each growth media variant (Fig. 6).

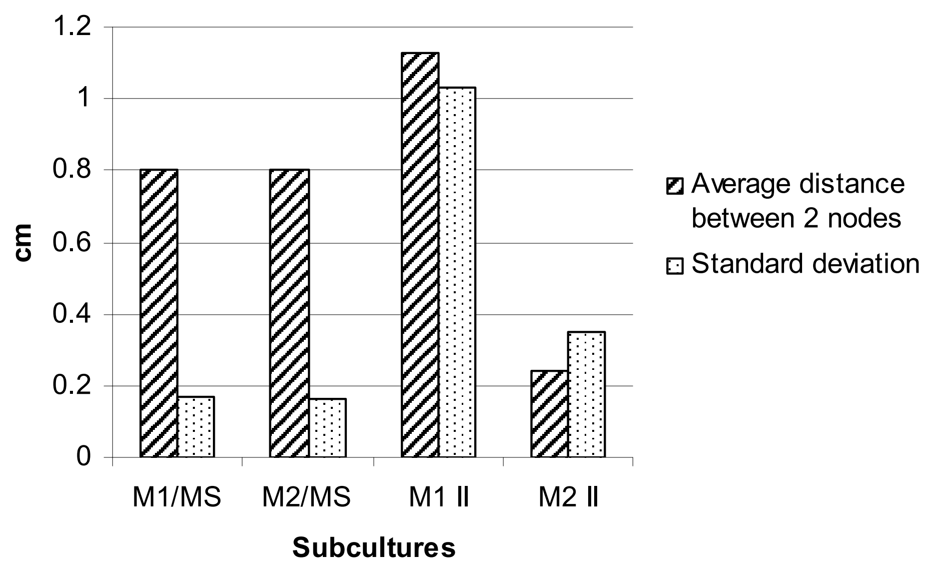

Figure6. Subculture I transferred on MS growth media without calcium pantothenate. The average distance between two nodes and standard deviation compared between the regenerated plantlets on MS growth media with and without calcium pantothenate.

Figure7. Regenerated plantlet on MS growth media without calcium pantothenate – root

Conclusions

The plantlets with calcium deficiency symptoms from Solanum tuberosum cv. Santé, cut and inoculated on MS growth media with calcium pantothenate were more uniform, leaves with normal aspect, root very well developed. After more subcultures on MS growth media with calcium pantothenate the average distance between two nodes reduced, but the best results were obtained when the cuttings were transferred on growth media without calcium pantothenate. We suspect that after more subculture the calcium is deposited like calcium oxalate (crystals) and has no effect on the future development of the plantlet. After the third subculture on MS growth media with calcium the plantlets developed well, but the growth was very slow. We suspect that to much calcium added to the growth media inhibits the plant growth.

In accordance with this results we recommend that plantlets from cv. Santé which present calcium deficiency, before the *ex vitro* acclimatization to be inoculated on MS growth media with calcium pantothenate.

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EFFECTS OF SOIL NITROGEN SUPPLY ON TUBERIZATION AND TUBER DEVELOPMENT IN PLANTS GROWN FROM MINI-TUBERS

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Introduction

Minitubers, small potato tubers grown in hydroponics, are increasingly being used in potato crop propagation but generally produce plants with low tuber numbers compared to plants grown from normal potato seed (Hills 2006; Ranalli *et al.* 1994). In Tasmania, Australia, these tubers are planted as the first of usually four field generations of seed potato. The number of tubers and the uniformity of tuber size produced in this first seed generation therefore impact significantly on seed quantity and quality by the time the fourth generation is grown.

Nitrogen has been shown to influence tuberization and tuber development. A number of hydroponics experiments by Kruass (1978; 1980; 1985) showed that nitrogen could delay and even inhibit tuberization. Early work by Werner (1934) suggested that tuberization was also delayed in the field by high nitrogen application, however O'Brien, Allen et al. (1998) suggested that Nitrogen in unlikely to effect the time of tuberization but is important in maximising tuber set.

Manipulation of nitrogen supply in hydroponics is common in the production of minitubers. Because first generation seed potato crops are grown over small areas of land, intensive management of the crop using treatments similar to those in hydroponic systems is possible. This paper reports on initial glasshouse trials examining effects of nitrogen availability on tuber formation, and forms part of a larger study conducted to find techniques to improve seed potato yield and uniformity in first field generation seed potato crops grown from minitubers.

Materials and Methods

Trial A

Trial A was a completely randomised 2x2 factorial design replicated 20 times (Table 1). Plants were grown in 10 litre pots and received four 220 ml applications of nutrient solution each day containing 100 ppm of NO3.

The constant N treatment involved a high nitrogen supply throughout the period of the trial while the leaching treatment involved a constant high nitrogen supply up to tuberization where the pots were leached with a total volume of 3.4 litres of water per pot over 2.5 hours. The nutrient solution was then applied without nitrogen for four days after which nitrogen supply was returned.

Table 1: Trial A treatments

Treatment	Soil	Nitrogen
1	Sand/Perlite (70/30)	Constant
2	Sand/Perlite (70/30)	Leached at tuberization
3	Clay (Red Ferrosol)[1]	Constant
4	Clay (Red Ferrosol)[1]	Leached at tuberization

[1]The clay soil used was a Acidic, Mesotrophic, Red Ferrosol; medium, non-gravelly, clay loamy/clayey, very deep (Isbell 1996)

Assessments

Plants were destructively sampled 14 days after the treatment was applied and tuber mass and diameter was measured. Soil samples were taken at the completion of the leaching treatment and analysed for mineral nitrogen.

Trial B

Trial B was a randomised block design replicated 5 times. Treatments consisted of a high and low nitrogen treatment in which each plant received four 220 ml applications of nutrient solution containing 100 and 10 ppm of nitrate respectively.

Assessments

Plants were sampled at ten times: 10, 14, 18, 22, 26, 30, 34, 38, 42 and 70 days after emergence and tubers were individually measured. Soil samples were taken at each harvest and analysed for major nutrients.

Statistical Analysis

Analysis was carried out using analysis of variance.

Results and Discussion

Rapid nitrogen reduction at tuberization

Average tuber size was doubled by rapidly reducing nitrogen levels in the Red Ferrosol soil when plants were harvested 14 days after the treatment was applied. The increase in tuber mass was evident with a significant reduction in tubers less than one gram and an increase in tubers in the larger size ranges (Figure 1). Jackson (1999) reports that the effect of nitrogen on tuberization and growth may be controlled by two possible mechanisms. The first suggests high nitrogen levels alter concentrations of phytohormones, increasing gibberellin levels and lowering abscissic acid, restricting tuber initiation and growth. The second theory is that the ratio of carbohydrates to nitrogen is important with a high ratio of carbohydrate promoting the formation of storage organs and high nitrogen promoting vegetative growth. It is therefore possible that the reduction in nitrate levels in the soil from 158 mg/kg in the unleached treatment to 51 mg/kg in the leached treatment increased the stimulus for tuber growth.

It was hypothesised that reducing nitrogen levels at tuberization may increase the number of tubers initiated due to the potential increase in resource partitioning to the stolons, however no effect on tuber number was measured. O'Brien, Allen et al. (1998) stated that sufficient nitrogen supply to achieve maximum ground cover during early crop development will provide the best tuber set. This theory is in line with the glasshouse results since both treatments in this trial received ample nitrogen supply throughout growth apart from the 4 day period at tuberization in the leaching treatment.

Although no difference in tuber number was measured 14 days after tuberization, the nitrogen leaching treatment may reduce the likelihood of tuber resorption. This process occurs later in plant development and can result a 23 to 44 % reduction in the number of tubers at final harvest (Ewing and Struik 1992). As tuber size increases so does their sink strength and this reduces the likelihood of them being resorped (Engels and Marschner 1986).

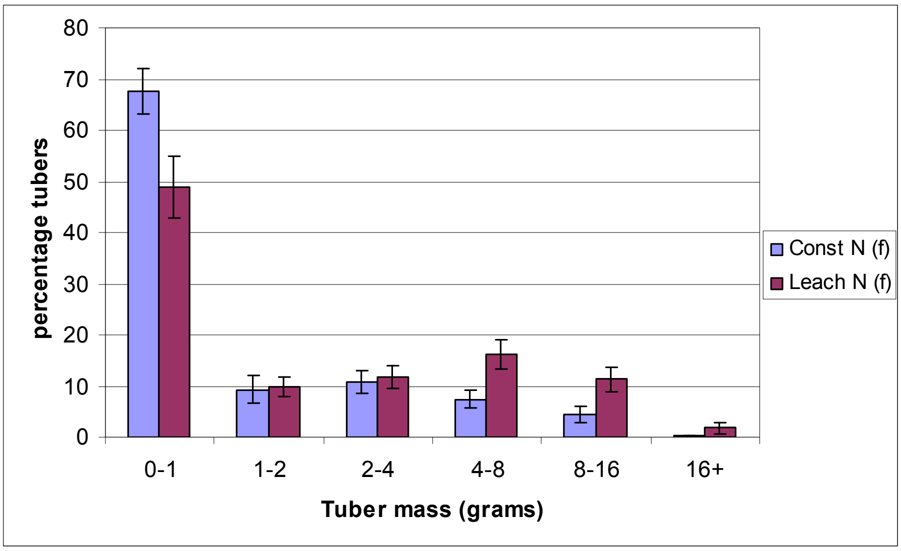

Fig. 1. Percentage of tubers in size catagories. Error bars represent the standard error of the mean.

High versus low nitrogen

Tubers were observed approximately four days earlier on plants receiving the high nitrogen levels compared to low levels (graph 1). These results show a trend similar to that found by Krauss (1978) and Werner (1934). Since the delay in timing of tuberization due to high nitrogen was relatively short, it was unlikely to have a significant detrimental impact on yield.

Plants receiving the high nitrogen treatment produced significantly more tubers per stem (Figure 3). This result further confirms the findings of O'Brien, Allen et al. (1998) that maintaining adequate nitrogen supply to plants in early stages of development is required to maximise tuber set. This appears to contradict the theories discussed by Jackson (1999) that high nitrogen inhibits tuberization and growth, however because nitrogen was supplied at low levels throughout the length of this trial, as opposed to only being lowered around the time of tuberization, vegetative growth was restricted reducing the photosynthetic capacity of the plant and in turn the level of carbohydrates supplied to the tubers.

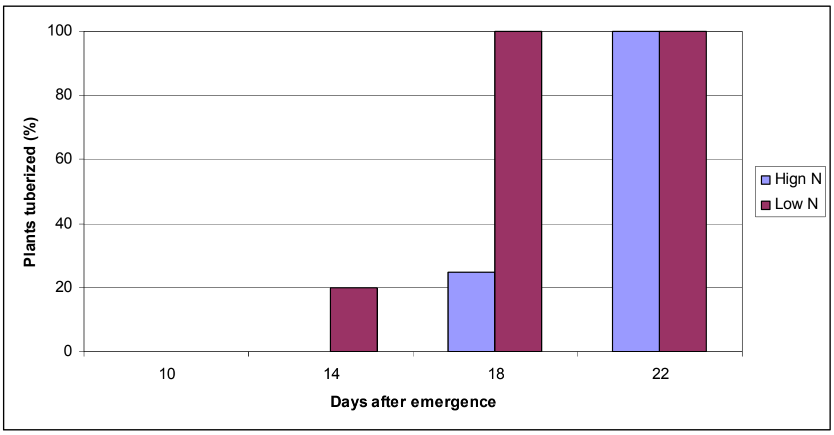

Fig. 2. Percentage of plants with tubers present at the first four sampling times.

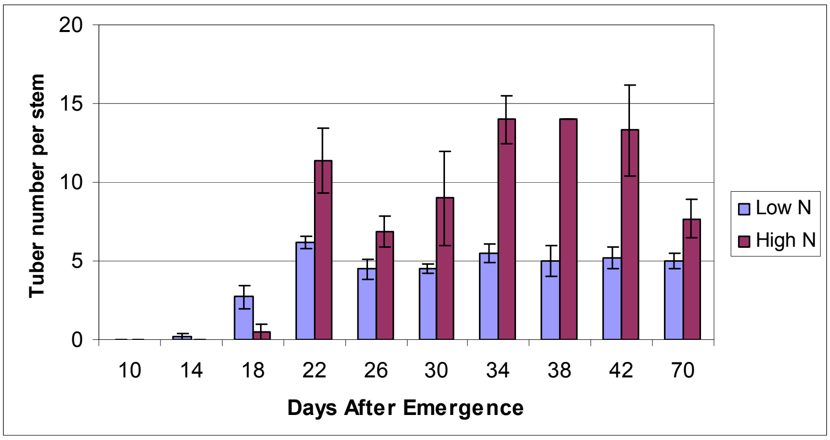

Fig. 3. Tuber numbers per stem at each sample date. Error bars represent the standard error of the mean.

Summary

Nitrogen was found to be a potential tool in manipulating tuber development in small, highly controlled, production systems. Reducing nitrogen availability at tuberization may provide a technique of increasing the number of tubers on the plant that will grow to marketable size.

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IN VITRO SELECTION FOR OSMOTIC TOLERANCE IN POTATO

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Under the circumstances of climatic changes that occurred more intensely during the last period, obtaining of new biological forms, i.e. varieties with increased resistance to draught and acidity of degraded soils, represents the ameliorators' main target.

Continuously increasing abiotic stress phenomena, as soils draught, acidity and salinity will determine a change in the agricultural lands configuration and will ask for creating and cultivation of varieties with resistance or with higher tolerance to these phenomena.

The National System for Soil Monitoring has shown that about 7.1 million hectares of agricultural are affected by "frequent draught" as major restrictive production factor.

Podzol soil (characterized by a low degree of natural fertility, due to their physical, chemical and biological properties) represent about one quarter of Romania's arable land, especially in Transylvania and Moldavia. In close connection to podzolisation is the occurrence of acidity within the soil top layers, by removing the bases from the primary minerals and then from the argilo-humic complex. Higher acidity of podzolic soils, with negative effects on cultivated plants, is accompanied by a deficit in nutrients, this resulting in a low fertility of these soils.

Due to the high percentage of clay, acid soils can store, even during the wet seasons, only a small amount of the precipitations water, thus water reserve being insufficient for long term.

Cultivation of potato valuable varieties, adapted to pedo-climatic conditions and customers' demands represent the main road for yield increased, together with observance of cultivation technologies. The varieties resistance to draught and unfavorable pedological conditions represents a fundamental element of yield stability in the areas where these phenomena take place.

Using a valuable germplasm and unconventional, biotechnological methods, such as "in vitro" selection, together with the selection performed inside greenhouses with controlled environment, and on field, under various climatic conditions, provide a good start for obtaining resistant potato varieties.

Material and method

In vitro cultures of plant tissues represent an important source of genetic (somaclonal) variability, being of great help in fulfilling and speeding the conventional amelioration programs (*Balan and Sand*, 2002). Although at the beginning *in vitro* cultures of plant tissues have been used for clonal micro-propagation, it has been noted that, under certain circumstances, they may represent valuable sources of genetic variability. This phenomenon is emphasized by the extension of the *in vitro* cultivation time, or when the regeneration system involves a calus phase. At the level of this tissue formation, during the cell differentiation and dis-differentiation processes, can take place certain changes in the structure or number of chromosomes, this determining the apparition of new genotypes.

The culture media we have used to induce the draught resistance in potato was the Murashige – Skoog one, supplemented with polyethylene glycol 6000 - PEG (Merck), that confer the possibility for *in vitro* induction of hydric stress. We consider this as an advantage compared to the classical method, by reducing the amelioration time and by giving the possibility of creating new varieties with increased draught resistance, compared to classical amelioration methodology.

Results and discussions

For obtaining potato plantlets we have considered the classical *in vitro* culture technology, starting from meristems cultures. After four weeks since inoculation, when potato plantlets have 4-6 internodes, they have been transferred into the rooting medium, where 20 g/L PEG was added.

The experiment consisted in *in vitro* rooting of 50 plantlets on a PEG-supplemented medium, compared to a standard of 50 plants cultivated on a medium without PEG.

After four weeks of *in vitro* cultivation, all plantlets have been transferred to pots, and leaves changes were analyzed.

Microscopy has shown that size and shape of the epidermal cells from the leaves surface of the two lots are different. The plants cultivated in PEG-added medium presented smaller epidermal cells, compared to those rooted on medium without PEG.

In what stomata shape and size concern, in the plants cultivated on PEG-added medium these have had an irregular pattern, small ones alternating with bigger ones, compared to the standard ones, that have had big, ellipsoidal stomata.

Monitoring the survival rate of the plants within the experiment we have noted that, after six weeks of *ex vitro* cultivation, from the plants cultivated on PEG added medium, 35 of them have survived (i.e. 70%), compared with 29 plants from the standard medium. The table below presents the data of the acclimation process:

Acclimated plants rate

	Acclimated plants rate, %			
	After 1 week	After 2 weeks	After 4 weeks	After 6 weeks
Plants on PEG-added medium	50	42	39	35
Plants on standard medium	50	41	38	29

These results being encouraging, we are determined to pursue our researches, differentiated on certain potato varieties and with various amounts of PEG.

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EFFECTS OF AGE AND PRE-TREATMENT OF TISSUE-CULTURED POTATO PLANTS ON SUBSEQUENT MINITUBER PRODUCTION

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Abstract

Tissue culture techniques are now routinely used to maintain and propagate pathogen tested potato (*Solanum tuberosum* L.) for field planting and for the production of microtubers *in vitro* or minitubers in greenhouses (Lommen and Struik, 1994). Environmental conditions, namely a short day photoperiod and cool night temperatures, can be used to induce tuberisation in potato plants (Struik and Ewing, 1995 and Jackson, 1999). Since one of the important factors that affect plant potential for tuber formation is the status of the potato plantlet at the end of the *in vitro* phase (Seabrook *et al*., 1995 and Tadesse *et al*., 2001) the possibility of preparing potato plantlets *in vitro* to produce more minitubers when planted in a greenhouse was investigated. This paper summarizes the effects of plantlet age and environmental conditions *in vitro* on plant morphology, tuber induction levels and minituber production.

Pathogen-tested plantlets of potato varieties Russet Burbank and Coliban were routinely multiplied every four weeks and cultured on standard MS medium (Murashige and Scoog 1962) with 30g/L sucrose and 7.0 g/L of agar. In addition to the standard (S) 4-week growth stage (temperatures of 22/18°C and daylength of 16 h), potato plantlets were also grown *in vitro* for up to 12 weeks using either standard or a combination of lower temperatures (16/10°C) during the hardening (H) stage and a chilling (C) stage (less than 12°C combined with short daylength of 12 or 8 h) to improve the tuberisation of tissue-cultured potato plantlets.

The age of tissue culture plants, in total during all stages *in vitro*, had a highly significant effect on the total number of minitubers produced per plant in a greenhouse. For Coliban yield increased from 2.50 to 3.89 minitubers per plant when the *in vitro* growth period was extended from 4 weeks to 12 weeks while for Russet Burbank the yield increased from 3.80 to 4.73 minitubers per plantlet. Lower temperature conditions used during hardening (H) and chilling (C) stages *in vitro* also induced potato plantlets to produce more tubers *ex vitro*. Overall, yield increased by up to 88% (2.50 vs. 4.72 minitubers per plant) in variety Coliban and up to 71% (3.80 vs. 6.50 minitubers per plant) in variety Russet Burbank when these two stages combined to condition potato tissue-cultured plantlets *in vitro* for the minituber production.

The standard (S) growth conditions *in vitro* produced plantlets with higher dry weights in total (38.60 mg/plantlet vs. 31.10 mg/plantlet) when compared with the growth conditions used during the hardening stage (H). A greenhouse experiment showed that a higher dry weight of potato plantlets at the end of *in vitro* growth phase was beneficial for the fresh weight of minitubers, but reduced the number of minitubers (5.52 minitubers/plant) compared to the plantlets with a lower dry weight (6.00 minitubers/plant). However, the fresh weight of minitubers was lower when plantlets with a lower dry weight at the end of *in vitro* growth phase were planted in a greenhouse. Treatments which combined both the standard temperature (22/18°C) and a lower temperature (16/10°C) regimes for 6 and 4 weeks *in vitro* produced plantlets that developed leaf area quicker, were first to reach full ground cover and maintained a higher plant leaf area size during the minituber production phase and resulted in the highest yield of minitubers (7.11 minitubers/plant).

The results presented in this report propose a possible way to adjust the conditions during *in*

vitro growth stage in such a way that the number and yield of minitubers produced per potato plant in greenhouse is increased. The higher yield of minitubers achieved by potato tissue-cultured plants *ex vitro* was a result of low temperature and the chilling stage conditions combined with the extended production phase *in vitro*. Further work is required to optimize treatments *in vitro* for different varieties.

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EFFECT OF POT SIZE, PLANTING DATE AND GENOTYPE ON MINI TUBER PRODUCTION OF MARFONA POTATO CULTIVAR

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This study was carried out to evaluate the effects of pot size, planting date and type of genotype on minituber production of Marfona potato cultivar (Solanum tuberosum L.) in greenhouse conditions. Four genotypes (M-129, M-128P, M-127P and M-124P) originated from virus free sprouts and a genotype of the same cultivar (Marfona) originated from apical meristem, in 3 sizes of pot and 3 planting date were investigated. The results showed that using larger pots of 3-liter has no advantage and smaller pots of 2-liter is not suitable for minituber production. Also, time of Nov 18 was the best of date for planting of potato in studied conditions and delay in date of planting reduced the minituber production. The reduction in number of minitubers and growing period was greater for the genotype M-129 compared with the other potato genotypes. Furthermore, higher numbers of minitubers were produced by the M-127P and M-124P genotypes and M-127P had the highest total weight of minitubers. However the number of minitubers per plant was higher for genotypes originated from meristem culture than genotypes obtained from sprouts. It seems that genotypes originated from potato sprouts are not as efficient as the apical meristem ones. On the other hand, later genotype showed more homogenous in growth rate and phenotype.

VIRUSLESS POTATO SEED PRODUCTION IN GEORGIA USING ELISA READER FOR VIRUSES CONTROLLING

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Biotechnological method is comparably new phenomenon in getting the seed potato. Reproduction system is different from the usual vegetation methods: 1. All the seed potato is from the culture tube, 2. Observation is made over all seed consignments to find out the diseases. The seed consignment starts from the clear tissue culture got from the culture tube. The maternal plants pass the most sensitive tests. As the sample is growing in the sterile area, and the tests are holding with a high degree of accuracy, the maternal plant is practically free from diseases. Using of Elisa Reader (LX-300 +IL/LX-1170 II) for the virus control in in vitro potato seed production nowadays is beneficial for our Biotechnology Center to strengthen our farmers who produce the virus less potato seed. Nowadays there is not a no virus free seed production in Georgia. Such products are bringing in by non governmental organizations (NGO) in small quantities (500 t.) and dispense them among the farmers. This is not enough for development potato production in Georgia. It also must be mentioned, that this potato seed can not adapt to the local climate conditions. If such potato has big harvest in its country, in Georgia it is much low.

STUDY ABOUT POSSIBILITIES OF REPEATED HARVESTING OF POTATO MINITUBERS RESULT FROM PLANTLETS IN GREENHOUSE

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Plantlets resulted from tissueculture laboratory planted in the vases with 25 cm in height and 30cm in diameter.Harvest times were:4weeks,7weeks and 10weaks after panting date which took place as B2,B3 and b4 in subplots.The B1 was as check(non-destructed harvest).A1 and A2 were Agria and Advanced clone No. 397007-9 took place in mainplots.We measured 16 agicultural traits.We used ANOVA and Duncan Tests which showed highly significant differences between B levels.B4 with three harvests(two non-destructive and one with destructive harvests)with 28.30tuber/vase took place first class.

EFFECT OF POTATO MINITUBER WEIGHT ON YIELD QUANTITY OF ADJECTIVES IN AGRIA AND OMID-BAKSH CULTIVARS

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This study was conducted to investigate the effect of potato cultivar and minituber weight on yield and some traits in greenhouse of Mohaghegh-Ardabili University at 2006. All adjectives affected significantly by minituber weight, exception plant height. Mean comparison showed that Omid-Bakhsh cultivar advantaged on Agria cultivar and optimum minituber weight was found at 200-250mg and 1g rang.

STUDY EFFECT OF PLANTING BEDS FOR PRODUCING POTATO MINITUBERS UNDER GREENHOUSE CONDITIONS

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In vitro plantlets of three potato cultivar planted in to eight different groving medium in black plastic bags in a greenhouse.Greater numbers and weight of minitubers were produced by the 397009-9 clones and Agria cvs as compared to cvs Marfona.The Biolan:Perlite (2.5:1)produced significantly yield (kg/m2)and number of minituber as compared to other planting beds.

EFFECT OF PLANTING DENSITY AND SIZE OF POTATO MINITUBERS ON YIELDS OF THE PRODUCED POTATO SEED TUBERS

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This study was conducted to determine the best density and minituber size in Agria potato cultivar for 2 years (2005 - 2006)in Ardebil Agriculture Research Station.The result showed that planting density and size of minitubers were significantly influenced tuber yield,mean yield of single plant,and seed sizes categories.Economically evaluation showed that the highest tuber yield was found in 75*12cm planting density with 44.534 t/ha and 11-20 gr minitubers seed sizes with 46.3 t/ha respectively.

STUDY ABOUT EFFECTS OF MINITUBER SIZE AND DENSITIES ON YIELD AND ITS COPONENTS OF AGRIA AND ADVANCED CLONE 397007-9 TUBERS

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In this study we grouped minitubers of agria and advaced clone no.397007-9 in three diameter sizes,5-20mm,20-35mm and 35-55mm and planted in the three desities,15cm,20cm and25cm on the planting rows.The statistical design was Split Factorial,that variety,density and minituber sizes as A,B and C levels main and subplots(B*C)respectively.We found that A2,C2 and B1 with10.15, 9.82tuber number/plant and 41.79t/ha took place in the a,a and b class respectively.

EFFECT OF GENOTYPE AND POT SIZE ON MINITUBER YIELD IN POTATO (SOLANUM TUBEROSUM L.)

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The potato (Solanum tuberosum L.) is a vegetable crop of major economical importance world -wide. This research was carried out with purpose of investigating the production of virus free plantlets from meristem culture in two cultivars of potato. The objective of the present research is study of genotype (2 commercial cultivars of S. tuberosum Marophona and Agria), substrate combination including 3 planting bed soil/sand/perlite (1:1:1), turb/perlite (1:1) and turb/rice hull/perlite (1:1:1), pot size with 19 cm (large) and 13 cm (small) diameters and their interactions on number and total weight of minituber.

PHYTOPLASMA & ZEBRA CHIP

STOLBUR PHYTOPLASMA INDUCED DISEASE OF POTATOES GROWN IN ROMANIA - I. BIOLOGY OF AND POTATO RESISTANCE AGAINST THE POTATO STOLBUR PHYTOPLASMA

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Introduction

Phytoplasmas are wall-less and non-helical bacteria of the class Mollicutes. Potato stolbur is caused by phytoplasmas of the stolbur (16Sr-XII-A) group and mainly transmitted by the planthopper *Hyalesthes obsoletus,* a southern European, xerothermic species.

The potato stolbur phytoplasma has quarantine status in the European Union (Status: EPPO A2 list, Nr. 100, EU Annex designation II/A2). In Germany potato stolbur was first detected in Hesse in 2006 and immediately eradicated.

A noteworthy loss of yields due to potato stolbur is not to be expected in the near future. However, growers should keep an eye on the further development of the disease because of the quarantine status of potato stolbur and its adverse affects on tuber quality.

Potato stolbur is detected in the Banat region of Romania every year with different intensity. Therefore, field experiments were carried out there in 2007 to gain more knowledge about hosts, vectors, symptoms of the disease, stolbur resistance of potato varieties, and change of potato tuber components caused by potato stolbur phytoplasma.

The experiments should give an answer to these questions:

1. Is the planthopper *H. obsoletus* the only vector that transmits stolbur phytoplasma?
2. Are there more natural host plants for stolbur phytoplasma beside *Convolvulus arvensis*?
3. Are there differences in resistance of potato cultivars against potato stolbur?

Material and Methods

The experiments were carried out by the company FHE-Handel-SRL, Sannicolau Mare, near Timisoara, Banat, Romania. The soil was a loess/clay of high quality. The average maximum temperatures between April and August were 20.3 °C, 24.6 °C, 29.4 °C, 30.7 °C and 29.2 °C, respectively. The average lowest temperature at night during the same time period ranged between 4.3 °C and 16.8 °C (4.3 °C, 12.0 °C, 15.1 °C, 16.0 °C, 16.8 °C).

To detect vectors for potato stolbur phytoplasma, leafhoppers were caught using either yellow traps or sweep nets and stored in ethanol. Leafhoppers emerging in higher numbers were identified according to genus and tested for infection by potato stolbur phytoplasma using PCR.

To detect further hosts for stolbur phytoplasma beside *C. arvensis*, plants from the experimental plot and the adjacent environment were collected for examination. The plants were tested for stolbur phytoplasma infection (PCR) and, if tested positive, the genus was identified.

Ten potato varieties - Salome, Bintje, Agria, Hansa, Belana, Layla, Lady Clare, Pirol, Saturna, and Hermes - were tested to evaluate their specific resistance against potato stolbur. The field experiment started on April 24th. A randomised block design with 4 replications was set up. 100 potato tubers per cultivar were planted in a plot of 26 m^2. Ten and twelve weeks after planting disease symptoms were observed and recorded. The potatoes were harvested on August 6th and 7th. While the potato tubers were checked for potato stolbur phytoplasma using PCR, the tubers were also divided into three groups: healthy tubers, tubers with minor symptoms, and tubers with severe symptoms. Lastly, the sugar content of tubers was analysed.

Results

Vectors

Whereas the analysis of the yellow sticky trap catches and live-catches showed no traces of *H. obsoletus, Reptalus* spp. (Auchenorrhyncha: Cixiidae) were detected in high numbers. On the 20 yellow sticky traps exposed from 05-07 to 19-07-2007, 330 specimens of *Reptalus* spp. were counted. During the time period of 20-07 to 06-08-2007, a total of 142 Auchenorrhyncha were caught on 11 yellow sticky traps. Twenty of them were identified as *Reptalus* spp. A sample of 10 *Reptalus spp*. obtained as live-catches was tested using PCR. Three planthoppers proved to be infected by phytoplasma.

Hosts

C. arvensis was evaluated to be the main host for *H. obsoletus*. Presumably, the pathogen overwinters in this perennial plant. Further perennial plants tested for phytoplasmas (e.g. *Cirsium arvensis*) were pathogen-free. Only one plant of the species *Carduus crispus* was infected by phytoplasma. This supports the assumption that phytoplasmas were transmitted from *C. arvensis* to annual plants during the vegetation period. Following plants had phytoplasma infections: *Datura metel, Amaranthus, Sonchus arvensis, Lathyrus sp., Epilobium spp.* and *Solanum nigrum.*

Resistance

The plants were evaluated for disease symptoms on July 3rd and 20th. Two to three leaves presenting disease symptoms were taken from each cultivar at the beginning of July. For Salome, Pirol, and Bintje stolbur-phytoplasma infection could already be verified at that time using PCR.
No PCR was performed with the leaves collected on July 20th. With the exception of the cultivars Agria (infestation: 27 %) and Leyla (infestation: 67 %), approximately half of the plants showed disease symptoms at that time. In parts, the plants had withered. Agria, however, proved to be still vital to a large extent.

All tubers from 10 plants of the first row (beginning with the fourth plant) of each replication were harvested. The first analysis of the tubers consisted of a visual counting of the three classes (healthy, minor, and severe symptoms (gummy)). No direct correlation between the visual symptoms of the plants and the percentage of diseased tubers existed. Though Pirol, Belana and Hermes yielded approximately 50 % of gummy tubers of both classes, Agria also presented a 50 % infestation of the tubers. Salome, Hansa, Lady Clare, Bintje and Saturna presented only 30 %-40 % diseased tubers. Thus according to the visual evaluation, Lady Clare and Saturna were relatively best qualified for crisping.

Changes of potato tuber composition caused by potato stolbur phytoplasma

Regarding the sugar content of the tuber, a direct correlation between the increasing disease development and the increasing sucrose content was ascertained. Under the influence of high temperatures during baking, this sugar may break down into fructose and glucose. These reducing are responsible for the non-enzymatic browning (Maillard reaction) of the crisps.

Conclusions

Stolbur is a serious potato disease. The effects on yields have been demonstrated in this study, particularly the proportions of tubers of inferior quality and increasing sucrose content, which leads to problems in further processing of the tubers.

Further investigations are necessary to explain the epidemiology of potato stolbur in the Banat region. While *C. arvensis* was found to be a common alternative host of the pathogen like anywhere else in Europe, the vector *H. obsoletus* has not been found so far. The presence of Cixiid planthoppers of the genus Reptalus, also suspected to be vectors of stolbur phytoplasma elsewhere, and the detection of stolbur phytoplasmas in some of these specimens give rise to further investigation of the role of this species in potato stolbur epidemiology in Romania.

POTATO STOLBUR PHYTOPLASMA INDUCED DISEASES OF POTATOES GROWN IN ROMANIA - II. LOW MOLECULAR WEIGHT CARBOHYDRATES

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Introduction

Phytoplasmas are bacteria-like organisms which lack cell walls and are solely enclosed by a three-layered plasma membrane. In contrast to viruses, phytoplasmas have an own metabolism which is, however, considerably reduced. Phytoplasmas are currently classified in the Mollicutes class that are categorised by molecular genetic differences on 16S rDNA-level. Accordingly, potato stolbur belongs to the 16 Sr XII-(stolbur)-group.

Symptoms induced by potato stolbur phytoplasma are yellow discoloration with curling up of the young leaves. Furthermore, tubers may appear as areal stolons and may become soft (gummy). Last but not least, tuber composition changes.

Processing of stolbur infected tubers resulted in dark coloured crisps, which cannot be accepted by both industry and consumers, because discolouration is accompanied by a bitter taste. Melanoidins (brown pigments, generated in the Maillard reaction) are also important contributors to inflammation and disease states. Therefore, industry has rejected those infected potato lots.

Material and Methods

Potato tubers from six different cultivars (Agria, Bintje, Hermes, Lady Claire, Pirol, Saturna) were harvested in the Banat region of Romania in 2007 (4 replications), transported to Detmold, Germany, and subdivided into healthy, minor infected and severe infected samples. Tubers were analyzed after freeze-drying and grinding to its sugar content by an enzymatic method, to find out any reasons for the discolouration of products. In addition to these analyses a sample from another cultivar was separated into two subsamples (minor and severe symptoms). Next to whole tubers, distinct parts of the tubers were prepared by using a cork drill (bud end, core, stem end).

Results and Discussion

Table 1 indicates a dramatic change of the biochemical pathway within infected tubers, because the fructose concentration was on a very low level. At the same time, the sucrose level increased significantly (threefold from healthy to severe infected tubers). This indicates an enhanced potential for Maillard reaction products because sucrose may undergo a thermal cleavage toward reducing sugars.

***Tab. 1:** Dry matter and low-molecular weight carbohydrates in potato lots of six cultivars, partly infected with stolbur (average values ± std. dev.; n = 4; FW: fresh weight)*

Object	Dry matter [%]	Glucose [mg/100 g FW]	Fructose [mg/100 g FW]	Sucrose [mg/100 g FW]	Reducing sugars [mg/100 g FW]	Total sugars [mg/100 g FW]
Healthy tubers	23.9 ± 2.29 b	34.1 ± 35.4 a	7 ± 10.7 a	308 ± 55.8 b	40.8 ± 45.3 a	349 ± 69.3 b
Minor symptoms	23.8 ± 2.28 b	45.3 ± 49.5 a	8.2 ± 10.8 a	435 ± 183 b	53.2 ± 59.6 a	488 ± 203 b
Severe symptoms	26.2 ± 2.46 a	47.3 ± 45.9 a	8.1 ± 9.0 a	975 ± 318 a	55.4 ± 53.6 a	1,030 ± 340 a

Different letters indicate a statistically significant difference (P > 0.05) (Student-Newman-Keuls-Test)

Reducing sugars increased especially in the cultivar Bintje (tubers with severe symptoms), whereas the other cultivars remained most stable. Sucrose concentration increased significantly in tubers with severe symptoms (gummy-like texture). Bintje had the highest sucrose level, followed by Saturna and Hermes. They all exceeded levels above 1000 mg sucrose/100 g FW. Lady Claire had the lowest sucrose concentration in stolbur affected tubers (Table 2).

***Tab. 2:* Sucrose content [mg/100 g FW] of the three quality classes of the investigated cultivars**

Object	Bintje	Agria	Lady Claire	Pirol	Saturna	Hermes
Average	692 ± 441	500 ± 346	410 ± 204	487 ± 256	691 ± 413	757 ± 419
Healthy tubers	328 ± 3.69 b	234 ± 58.6 b	297 ± 15.2 b	311 ± 25.9 b	356 ± 54.3 b	367 ± 56.6 a
Minor symptoms	516 ± 50.4 b	333 ± 83.4 b	292 ± 33.4 b	352 ± 87.8 b	544 ± 78.1 b	722 ±452 a
Severe symptoms	1,233 ± 320 a	932 ± 220 a	642 ± 209 a	798 ± 188 a	1,174 ± 351 a	1,182 ± 53.7 a

Different letters indicate a statistically significant difference ($P > 0.05$) (Student-Newman-Keuls-Test)

Next to the absolute concentration of constituents also the distribution within tubers was investigated. Tubers with severe stolbur symptoms had a higher dry matter concentration than tubers with minor symptoms. In both cases, the core region had a lower dry matter concentration than bud or stem end. Glucose and fructose concentration was highest in the stem end region, whereas sucrose concentration was uniform over the different tuber regions (Tab. 3).

***Tab. 3: Dry matter and low-molecular weight carbohydrates in potato tubers with stolbur symptoms, separated into sections*.**

Stolbur	Section	DM [%]	Glucose [mg/100 g FW]	Fructose [mg/100 g FW]	Sucrose [mg/100 g FW]
Minor symptoms	Whole tuber	25.9	52	19	1,011
	Bud end	26.1	206	69	1,165
	Core	22.5	21	12	972
	Stem end	26.2	110	20	871
Severe symptoms	Whole tuber	30.9	51	17	2,550
	Bud end	33.6	252	149	2,325
	Core	28.2	38	17	2,451
	Stem end	34.6	89	13	2,485

Conclusion

Potatoes with stolbur symptoms had significantly higher sucrose levels than the control, equally distributed within tubers. These tubers are not qualified for any processing. It will be a challenge to develop tools to separate infected tubers from single lots. This seems to be the only way to prevent a non-acceptance by the factories.

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ADDENDUM

EFFECTS OF COMPOST APPLICATION ON THE DEVELOPMENT OF SILVER SCURF (HELMINTHOSPORIUM SOLANI) IN POTATOES

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Summary

The development of silver scurf (*Helminthosporium solani*) mainly takes place during the storage period. However, the main primary infection sources for newly harvested tubers are the mother tubers planted in spring producing silver scurf spores on existing (and expanding) lesions. These spores are transferred to the newly formed tubers by means of passive transport, or can infect the new tubers during harvest.

Survival of silver scurf spores may be influenced by soil processes, such as the presence of antagonists, or the production of plant metabolites with a anti-fungal activity such as isothiocyanate, released from cabbage crop residues. Application of other composts may also reduce the survival rate of the spores in soil, and thereby reduce the silver scurf infection on daughter tubers at harvest.

This hypothesis was tested in experiments in 2005 and 2006 with different types of compost, among which cabbage-based compost. Compost applied at planting time had no reducing effects on the silver scurf infection on daughter tubers. It is concluded that the only way compost might reduce silver scurf is when with the compost specific antagonists of silver scurf are applied. Research to optimize this approach is started in 2008.

Introduction

Silver scurf, *Helminthosporium solani*, is a major problem in organic as well as in conventional potato production Mostly considered a storage disease, the source of the problem lies in the seed. Silver scurf on an infected seed tuber starts to sporulate after planting, most intensively at the border of the lesions. The spores are transferred to daughter tubers by means of passive transport in the soil (precipitation), but infection of daughter tubers in the soil is only of limited importance. At the end of the growing season, when the tubers start to ripen, and form a real skin, infection by silver scurf becomes visible. Furthermore, daughter tubers can be infected when spores get established on the potato skin during harvest, transport and store loading.

In the course of time, the spores die off in the soil. After 70 days in the soil, only 1 % of the spores survives. The earlier the tubers are harvested, the more surviving spores of silvers scurf may still be present. Enhancing the process of dying off of the spores is therefore an important aspect in reducing the damage by silver scurf.

It is not exactly known which processes influence the dying off of the spores. In storage facilities spores of silver scurf can survive for a long time without the presence of potatoes. It is therefore plausible that soil processes influence the survival rate of these spores in the soil. This may be by abiotic processes (temperature, humidity, etcetera), or by biotic processes (disease suppression by soil life).

In the literature disease suppression in the soil is defined as “a soil in which the pathogen does not develop or maintain itself, or develops but does not cause any damage, or develops and causes damage for a limited time period, after which the disease becomes less important, despite the fact that the pathogen can survive in the soil.”(Baker & Cook, 1974). Disease suppression can be general or specific. General disease suppression is caused by the sum of the activity of the total microbial biomass, while specific disease suppression is caused by the activity of one or some populations of antagonistic organisms (Stone et al., 2004).

There are little reports about disease suppression of silver scurf in potatoes. General disease suppression can be enhanced by amendments of organic matter and stimulation of soil life in general. One of the means to do this might be the application of compost. Different types of compost are available. Compost can be relatively rich in nitrogen on the one hand, or in carbon on the other hand. Nitrogen-rich composts have a relatively larger population of bacteria, and are supposed to feed soil life directly, while carbon-rich composts have larger populations of fungi, and are supposed to enhance disease suppressive properties of the soil.

In 2005 and 2006 we applied three (2005) or four (2006) different composts to potatoes in a pilot experiment and tested if the silver scurf infection on the daughter tubers was reduced. We focused on general disease suppression, and chose one carbon-rich and one nitrogen-rich compost as well as a cabbage-based compost which might produce plant metabolites with an anti-fungal activity.

Materials and methods

The experiment was laid out on two organic arable farms in Flevoland (The Netherlands) in 2005 and 2006 with potatoes of the variety Santé. Seed potatoes with two infection levels (low and high) were used in the experiments (see table 1). In 2005, the high infection level was obtained by chitting the seed; on farm 1 this did result in only a minor difference in seed infection. In 2006 seed with high or low infection was collected from 1 seed lot.

Table 1. Seed infection levels (% skin covered by silver scurf)

		Low seed infection	High seed infection
2005	Farm 1	22	25
	Farm 2	19	28
2006	Farm 1	4	37
	Farm 2	6	48

The potatoes were planted by hand. Crop management was according to the management of the commercial potato crops grown on the farms (see table 2).

Table 2. Crop management on experimental farms.

	2005		2006	
	Farm 1	Farm 2	Farm 1	Farm 2
Planting date	26 April	13 May	25 April	24 April
Defoliation	15 July	29 July	7 and 16 August	11 August
Harvest	2 August	23 August	23 August	31 August

Three (2005) or four (2006) different types of compost were applied, and compared to a variant without compost:

- *Household-waste-compost (HWC)*: relatively high N-contents, easily biodegradable, relatively high amounts of bacterial biomass, activating soil life processes
- *Green compost (GC)*: relatively high C- and lower N-contents, more difficult biodegradable, relatively high amounts of fungal biomass, stimulating soil suppressiveness
- *Cabbage-based compost(CBC)*: possible production of metabolites (i.e. isothiocyanate) with an anti-fungal activity.
- An *'enriched' green compost (EGC)*, to which two different micro-organisms were added (only in 2006).

The composts were applied when planting the potatoes, in an amount of 15 tonnes dry matter/hectare concentrated around the seed tubers (see photograph 1).

Photograph 1: Compost was concentrated around the seed tubers.

The fertilisation was not corrected for the differences in N applied with the different composts. The experiments were laid out as randomized block experiments with 6 replications.

Assessments

Composts. At the moment of application a sample of each compost was analysed for nutritional contents and for total and active fungal and bacterial biomass.

Silver scurf. Tuber samples were taken at harvest (50 tubers per plot) and assessed for the presence of silver scurf. Additionally, on three moments in 2006 (June 27, July 13, August 23) on farm 1 the daughter tubers of one plant per plot in three of the six replications were sampled and assessed for the presence of silver scurf. Tubers were dried, and stored at 4 °C until incubation. Before assessment for silver scurf, tubers were washed, and incubated for 10 days at 18 °C at a relative humidity of 90 % or more. After incubation the tubers were assessed for the percentage of the skin infected by silver scurf.

Results

Compost

Analyses of the composts are displayed in table 3.

Comparing HWC and GC, HWC was relatively high in N and more dominated by bacteria, while GC was more dominated by fungi.

CBC was relatively low in dry matter (44-58 %) and organic matter (5-10 %), when compared to the other composts (DM 59-69 %, OM 8-16 %). The amount of bacteria (only 2005) and fungi was relatively high.

Table 3 . Compostanalyses in 2005 and 2006.
N, P and K: gr per kg dry matter

	Household waste compost (HWC)		Green compost (CG)		Activated green compost (AGC)		Cabbage based compost (CBC)	
	2005	2006	2005	2006	2005	2006	2005	2006
Dry matter (%)	68,5	58,7	67,4	64,4		63,3	43,5	58,3
Organic matter (%)	15,7	7,90	12,8	10,90		12,89	9,6	5,37
N (g/kg dry matter)	10,93	6,32	6,95	5,76		7,61	9,35	3,69
P_2O_5 (g/kg dry matter)	4,69	9,16	2,78	8,86		7,14	8,33	11,32
K_2O (g/kg dry matter)	9,28	7,90	5,35	10,90		12,89	14,14	5,37
Total bacterial biomass (µg/g)	4599	5646	812	1749		2466	7060	2695
Active bacterial biomass (µg/g)	72.95	66.2	60.3	85.2		68.2	331.4	86.7
Total fungal biomass (µg/g)	470	340	302.5	503		936	1349.5	953
Active fungal biomass (µg/g)	13.1	11.9	13.85	22.2		56.3	65.75	29.9

Silver scurf infection of daughter tubers

Both in 2005 and in 2006 the application of compost resulted in the same, or in a higher infection level of the daughter tubers with silver scurf (table 4).

Table 4. Silver scurf infection of daughter tubers.
For comparisons within one year and one farm: values with different letters are significantly different from each other (5% level).

		No compost	HWC	GC	AGC	CBC
2005	farm 1	8,29 a	10,94 a	16,17 b		13,37 b
	farm 2	11.84 a	11.86 a	11.68 a		16.97 b
2006	farm 1	2,72a	3,97bc	3,22ab	4,55c	3,70abc
	farm 2	15.22a	17.75ab	20.73b	24.16c	17.12a

Conclusion

Neither in 2005 nor in 2006 did compost did reduce the amount of silver scurf on the daughter tubers. On the contrary, application of compost often enhances silver scurf infection of daughter tubers.

It may be concluded that the only way compost might reduce silver scurf is when with the compost specific antagonists of silver scurf are applied.

References

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DETECTION AND QUANTIFICATION OF LATENT SEED-BORNE INFECTIONS USING qPCR

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Many pathogens of potato can exist as latent infections on seed tubers that pose a risk to progeny crops if planted. Examples of such pathogens include, Polyscytalum pustulans (skin spot), Rhizoctonia solani (black scurf), and Colletotrichum coccodes (black dot). The use of real time PCR to detect and quantify pathogens on seed tubers to determine the risk of disease developing of progeny crops will be discussed.